

RELATIONSHIPS BETWEEN WATER QUALITY, SPECIES COMPOSITION,  
BIODIVERSITY AND ECOSYSTEM FUNCTION IN LAKES AND FLOODED  
PITS EXPOSED TO URANIUM MINING ACTIVITIES IN NORTHERN  
SASKATCHEWAN

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By

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## **ABSTRACT**

Uranium mining activities have the potential to impact aquatic systems through mine drainage (runoff) and the release of treated effluent into nearby watersheds. Such anthropogenic exposure can lead to elevated concentrations of metals and major ions, which may impact aquatic biota. Previous studies have looked at the effects of water quality on aquatic biota within flooded pit lakes and natural lakes that have been exposed to various mechanisms of mining exposure. However, the literature often only examines the effects of a limited number of contaminants on a limited number of species. Researchers have rarely looked at the effects of multiple contaminants on species composition, biodiversity and ecosystem function in aquatic systems. This study uses a multivariate approach to look for relationships between water quality (24 variables), plankton species composition and abundance, biodiversity (richness and evenness) and ecosystem function among lakes exposed to mining activities ( $n = 18$ ) and non-exposed reference lakes ( $n = 8$ ). Lake water quality data was used to cluster lakes into groups. Lake groups were then overlain onto multivariate ordinations derived from species composition-abundance data to determine if species composition was related to water quality. Ecosystem function variables included planktonic phosphorus cycling and planktonic respiration. The classified lake groups clustered well on ordinations derived from species composition-abundance data suggesting that relationships exist between water quality and plankton species composition. However, ecosystem function was similar among the majority of lakes and flooded pits despite differences in species richness, species composition and species abundance. Only a small number of aquatic systems had ecosystem function properties that were different from the majority of lakes and pits. These systems had the greatest concentrations of contaminants and had very low biodiversity (richness and evenness) compared to the other systems. Despite having differences in plankton species composition and species richness, all lake groups were functionally similar. This suggests that functional redundancy in species composition may be present in the majority of lakes and pits in such a way that ecosystem function is maintained.

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## **CHAPTER 1. INTRODUCTION**

Northern Saskatchewan contains some of the world's richest uranium deposits (Pyle et al. 2001). Uranium mining activities release treated effluent into nearby watersheds (Cogema 2000) and create runoff (Nyogi et al. 2001), elevating the concentrations of metals and salts in receiving lakes and flooded pit-lakes. These elevated metals and salts can act as aquatic stressors. Multiple stressors affect aquatic systems by altering water quality (Christensen et al. 2006; Pyle et al. 2001) and have the potential to impact biodiversity (Evans and Prepas 1997; Vinebrooke et al. 2004) and ecosystem function (Gessner et al. 2004; Kaneko et al. 2004; Morin and Mcgrady-Steed 2004; Morin 1995; Petchey et al. 2004). The degree of impact within aquatic systems is dependent upon the degree of exposure and the mechanism of exposure. Impact can be investigated at various levels. For example, exposure to uranium mining activities may have an impact on water quality, which may or may not translate into impacts on biodiversity and ecosystem function. Changes in water quality resulting from mining exposure are influenced by the proximity of receiving waters to mining activities. Contamination is typically greater near the source and decreases downstream (Lukin et al. 2003; Nyogi et al. 2001). This often results in different community structure near the source of the contamination compared to that farther downstream (Cattaneo et al. 2008).

Aquatic ecosystems respond to stress in numerous ways. For example, a number of ecological indicators (Xu et al. 2001) are typically associated with ecosystems that are exposed to chemical stressors (e.g. mining). Odum (1985) identified decreased species diversity, a greater proportion of r-selected species, selection for small bodied organisms, and decreases in (or extinction of) large bodied zooplankton in response to stress. Xu et al. (2002) found that chemical stress caused enlarged phytoplankton cell size, diminished zooplankton body size, decreased total zooplankton biomass, reductions in species richness and diversity, decreases in zooplankton:phytoplankton and macrozooplankton:microzooplankton biomass ratios, and a decline in structural



exergy. Structural exergy measures an ecosystem's ability to effectively utilize resources (Jorgensen 1992). High structural exergy is often found in more complex plankton food webs, which typically have greater diversity and exhibit more resource partitioning. These factors enable ecosystems to use resources more efficiently (Jorgensen et al. 1995; Xu et al. 2002).

Other studies have found that the observed sensitivity of phytoplankton communities to toxic chemical compounds depends on the community structure at the time of impact (Maraldo and Dahllöf 2004). These authors revealed that community sensitivity to two pyrethroid biocides varied seasonally in response to seasonal variation in total phytoplankton biomass and food web structure. High biomass and high species richness led to a decrease in community sensitivity by way of dilution effects. Dilution effects reduce toxicity due to increases in cell size and number (Lozano and Pratt 1994). In highly stressed ecosystems, sensitive species are depleted and there is often a shift towards a community dominated by only a few tolerant species (Admiraal et al. 1999; Vinebrooke et al. 2004). Shifts in taxonomic composition are often associated with reduced food web complexity (e.g. missing trophic levels) and reduced species richness (Havens and Carlson 1998).

Determining relationships between anthropogenic stress (e.g. mining activity), biodiversity, and ecosystem function is extremely complicated and has received recent attention (Gamfeldt et al. 2008). It has been proposed that maintaining high biodiversity is important for maintaining ecosystem function (Balvanera et al. 2006). However, other studies have suggested that single species monocultures perform certain ecosystem functions as well as more diverse mixes of species (Wardle et al. 1997), or that the effects of biodiversity on ecosystem function are largely related to the individual species lost (Cardinale et al. 2006). This implies that certain species are more important in controlling certain ecosystem functions and that the loss of such species has the greatest effect on function. Other theories suggest that most natural communities contain more biodiversity than is required to maintain function. In such a community, multiple species are present that can perform similar

ecosystem functions, resulting in functional redundancy (Loreau 2004). Functional redundancy provides insurance that the loss of a few species will not result in functional change (Folke et al. 1996). It is commonly predicted that changes in aquatic communities and food webs occur when ecosystems become stressed (Odum 1985).

Recent advances in biodiversity-ecosystem function theory can be largely attributed to two conferences that focussed on the merging of biodiversity research with that of ecosystem function. The first of these was held in Mitwitz, Germany in 1991 (Mooney 2002). This conference addressed two main questions: 1) Does biodiversity count in system processes over short- and long-term time spans and 2) how is system stability and resistance affected by species diversity, and how will global change affect these relationships (Schulze E.D. and Mooney 1994)? This conference sparked an enormous amount of theoretical and empirical research that centred around the effects of biodiversity, with a strong emphasis on the effect of species loss on ecosystem function. As a result, a second conference was held in Paris, France, in December 2000, which was entitled “Biodiversity and Ecosystem Functioning: Synthesis and Perspectives” (Loreau et al. 2002).

Despite the recent increase in biodiversity-ecosystem function research, the debate surrounding the effects of species loss, or addition, on ecosystem function has been on-going for more than 50 years (Loreau et al. 2002). At the core of this debate is the question, does diversity affect stability in ecosystem function, such as biogeochemical cycling, primary production, and respiration (Tilman et al. 2006a)? Early studies resulted in a mainstream concept that diversity leads to stability (MacArthur 1955; Odum 1969). MacArthur (1955) concluded that a large number of energy flow-paths through a food web are needed to maintain stability. This implies that as the number of trophic links and food web interactions increases (as would be the case with increased diversity) the more routes there are for energy to be transferred up the various trophic levels. According to MacArthur, this would have a stabilizing effect on the community, as shifts in population dynamics in more diverse food webs would

not have a large effect on overall community structure. Although MacArthur's study does not extend to full ecosystem-level processes, the issue of diversity and stability is certainly being addressed.

Odum (1969) addressed relationships between ecosystem properties and diversity in a discussion about ecological succession. In a table contrasting developmental and mature stages of ecological succession, Odum stated that the mature end-state of an ecosystem contains high species diversity (richness and evenness) and ecosystem stability (measured as resistance to external perturbations).

Studies opposing the theory of 'diversity leads to stability' emerged in the 1970's and 1980's (Loreau et al. 2002). For example, Gardner and Ashby (1970) used a modelling approach to determine if large, diverse systems were indeed stable. They concluded that stability decreased exponentially as the number of variables (a proxy for number of species) increased. Stability could be predicted by the connectance exhibited by the variables. Connectance was used as a measure of the interaction strength between each variable with all other variables. As connectance (complexity) increased, stability decreased, but if connectance remained low, then stability was predicted to be maintained, as the variables would have little interaction with one another. Support for this theory was provided by May (1972), again using a computer modelling approach. May's model predicted that a food web containing 12 individual variables (species) would have essentially zero probability of being stable. However, May also reported that if the 12 species were organised into 4 blocks of species, each containing 3 species, then stability could be attained. When ecological communities were organised into groups of species, rather than individual species, then diversity led to stability. This has striking similarities to what modern day research refers to as functional groups, or guilds (Fennel et al. 2007; Walker 1992). Therefore, as early as 1972, scientists were recognizing the importance of functional groups of species, rather than individual species.

Although the findings of May, Gardner and Ashbey provided alternative perspectives to biodiversity-function research, there were some shortcomings to

the research. For instance, the theoretical implications were based solely on mathematical modelling and had no empirical evidence to support or refute their conclusions. In response to this concern, McNaughton (1978) tested May's theories on grassland stands from the Serengeti National Park in Tanzania. He found that interaction strength and connectance between species decreased with increasing species richness, which was predicted by May (1972) to result in stability. McNaughton implied that communities are organised into blocks of species that interact among themselves, but interact little with other blocks and he used the term "guilds" to represent these blocks of species. These early studies led researchers to address the question, what kinds of biodiversity lead to stability (Walker 1992)?

Over the past two decades, biodiversity-ecosystem function research has shifted from populations to communities to ecosystems (Loreau et al. 2002; Tilman et al. 2006a; Walker 1992). New approaches to biodiversity-ecosystem function issues are much more holistic (Gin et al. 1998) than those of previous decades. There is still much debate over the functional role of biodiversity but there is a growing consensus that biodiversity has a stabilising affect on ecosystem-level properties (Dobson et al. 2006; Duffy et al. 2007; Tilman et al. 2006a; Walker 1992).

Historically, studies involving the effects of mine exposure on aquatic biota have only considered single contaminants and one or a few species. Such studies could rely on univariate statistical techniques for interpretation. However, within aquatic systems exposed to mining activities, it is unlikely that the biota will be exposed to a single contaminant. Rather, aquatic biota are subject to multiple contaminants across multiple species (Pyle et al. 2002). In these circumstances, it would be unrealistic to rely on univariate statistical procedures to provide reliable and interpretable results, and a multivariate statistical design is the appropriate choice (Maund et al. 1999). For these reasons, various multivariate techniques are applied throughout this thesis to search for relationships between lake (and pit) water quality, biodiversity and ecosystem function.

The use of multivariate statistics to study relationships between environmental stress, biodiversity and ecosystem function is becoming widespread in ecological studies (Clarke 1999; Maund et al. 1999). Some factors influencing the increased use of multivariate approaches are advancements in computer technology and an increased awareness of how to interpret multivariate results and apply multivariate techniques toward ecological community data (McCune and Grace 2002; Sparks et al. 1999). Common multivariate techniques found in the current ecological literature include Principal Components Analysis (PCA), Redundancy Analysis, Canonical Correspondence Analysis (CCA), Nonmetric Multidimensional Scaling (NMDS), Cluster Analysis and various permutations (Clarke 1999; Maund et al. 1999; McCune and Grace 2002; Van Den Brink and Ter Braak 1999). Proper matrix construction, measurement of appropriate environmental variables and selection of appropriate multivariate analyses ensures that multiple variables can be interpreted simultaneously across multiple species. As a result, meaningful relationships can be detected between environmental conditions, community structure and function. For example, (Gray et al. 1990) used NMDS to illustrate how benthic community structure changed across a distance gradient within an area impacted by offshore oil operations. Samples that were close in proximity (< 3 km) to the operations showed decreased species richness and strong dominance of tolerant species compared to samples > 3 km from the operations.

Multivariate statistical methods have gained acceptance in ecology because they effectively reduce the dimensionality of large data matrices involving many samples and many species. The reduced number of dimensions represents the strongest correlations in the matrix and preserves important information from the raw data (Bettinetti et al. 2000; Kenkel and Orloci 1986). Bettinetti et al. (2000) used NMDS to track variation in seasonal phytoplankton assemblage in sub-alpine Lake Como (Italy) that is undergoing a change in trophic state. Muyllaert et al. (2002) used CCA to determine if changes in seasonal bacterial communities were related to bottom-up or top-down controls.

In this thesis, relationships are explored among anthropogenic stressors (24 water quality variables), plankton species composition and abundance, biodiversity (species richness, and species evenness), and ecosystem function (represented as planktonic phosphorus cycling and respiration). Samples were collected over a three year period from lakes and flooded pit-lakes near uranium mine operations within the Athabasca Sand Basin, located in northern Saskatchewan, Canada. At each mine site, samples were collected from lakes that have been exposed in various ways to uranium mining activities (herein after called exposed lakes), as well as control lakes that have not been exposed (or have received a negligible amount of exposure) to mining activities (herein after referred to as reference lakes). There are various mechanisms of exposure and differing physical characteristics among the study systems (i.e. lakes vs. flooded pits). This provides an opportunity to explore potential relationships between exposure, biodiversity and ecosystem function across many aquatic systems within the same geographic location.

## **CHAPTER 2. CLASSIFICATION OF LAKES BY MULTIVARIATE ANALYSIS**

### **2.1 Introduction**

The effect of anthropogenic stress on aquatic systems has received considerable attention (Garcia-Villada et al. 2004; Lukin et al. 2003; Novotny et al. 2005; Nyogi et al. 2001; Pyle et al. 2001; Thomas and Liber 2001; Xu et al. 2001). Uranium mining activities have the potential to impact aquatic systems through mine runoff (Nyogi et al. 2001), and through the release of treated effluent into nearby watersheds (Pyle et al. 2001). Mine runoff and treated effluent often elevate the concentrations of metals and major ions above background levels and have the potential to impact aquatic biota (Nyogi et al. 2001; Pyle et al. 2001). In addition, open pit mining has historically been a common practice and, in many cases, has led to the creation of flooded pit lakes.

Many studies have looked at the water quality and limnological characteristics of pit lakes (Boeher and Shultze 2006) and natural lakes that have been exposed to various mining activities around the world. However, the literature often deals with the effects of only one or a few contaminants on aquatic biota (Garcia-Villada et al. 2004; Lukin et al. 2003). Seldom do researchers look at the effects of multiple contaminants and multiple mechanisms of exposure on biodiversity and ecosystem function of aquatic systems. My examination of multiple stressors on biodiversity and ecosystem function provides a more realistic method of exploring such relationships. This study compares such relationships across 26 aquatic systems. Due to a confounding number of variables, a multivariate statistical approach is necessary. Cluster analysis has been used to previously classify, or group, aquatic systems based on similarities (or dissimilarities) among several measured variables (Alexander et al. 2008; Kitner and Poulickova 2003; Vehanen and Aspi 1996).

Three mechanisms of exposure have been identified by industry for the study of lakes and pits. These include 1) natural lakes that have been exposed to uranium mining activities, 2) flooded mine pits that have been exposed to uranium mining activities, and 3) reference lakes (control lakes) that are

unexposed, or have received negligible amounts of exposure and, therefore, are not considered to be impacted. However, these categories may not be optimal due to the wide range in exposure mechanisms and the degree of impact from exposure. For example, some lakes and pits that are known to have been exposed are similar in water quality to reference lakes and might be grouped accordingly. This confounds the three aforementioned categories and a more objective classification method was required to group the lakes based on water quality. Multivariate Cluster Analysis is considered to be the best method to determine lake groups based on water quality data. This data was provided by Cameco Corporation and AREVA Resources Canada Incorporated. I then tested these new lake groups for significant group differences.

The first objective of this chapter was to establish appropriate lake groups, based on water quality, to use as the basis for analysis in subsequent chapters. Significant differences in species composition, biodiversity, and ecosystem function among lake groups may indicate impact from exposure to mining activities.

The second objective was to identify which water quality variables best classify, or represent, each group. For example, do exposed lakes contain higher concentrations of total dissolved solids (TDS) relative to the other lakes? The water quality variables that are representative of each lake group help determine the type of impact and the degree of impact by identifying which contaminants, if any, are present in high concentrations.

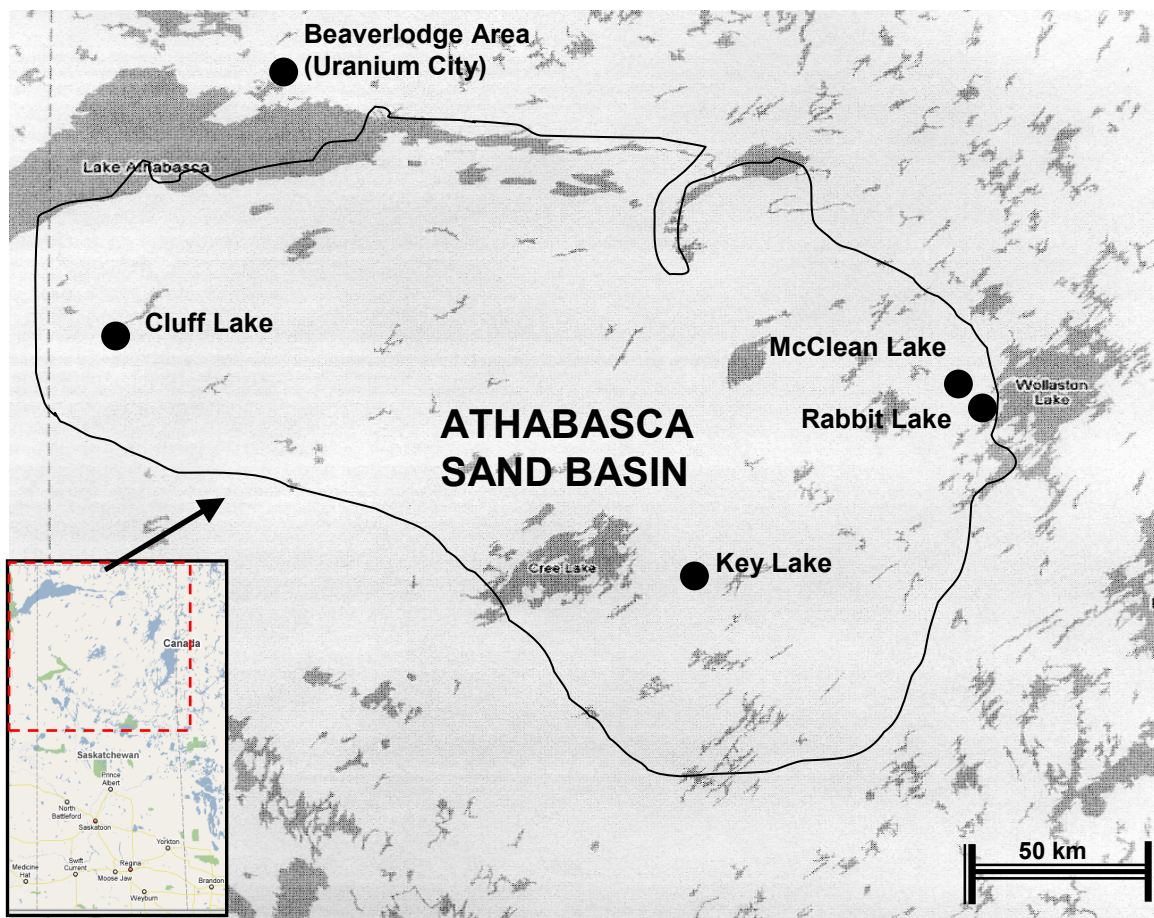
## **2.2 Study Sites**

The following sub-sections provide a detailed description of the lakes within the study areas and their associated mechanisms of exposure. The mine sites visited during my study are within the Athabasca Sand Basin, located in northern Saskatchewan (Fig. 2-1), except for the Beaverlodge Mine Area, which is located outside of the Athabasca Sand Basin. All study lakes are summarized by exposure mechanism (Table 2-1).



### 2.2.1 Beaverlodge Area

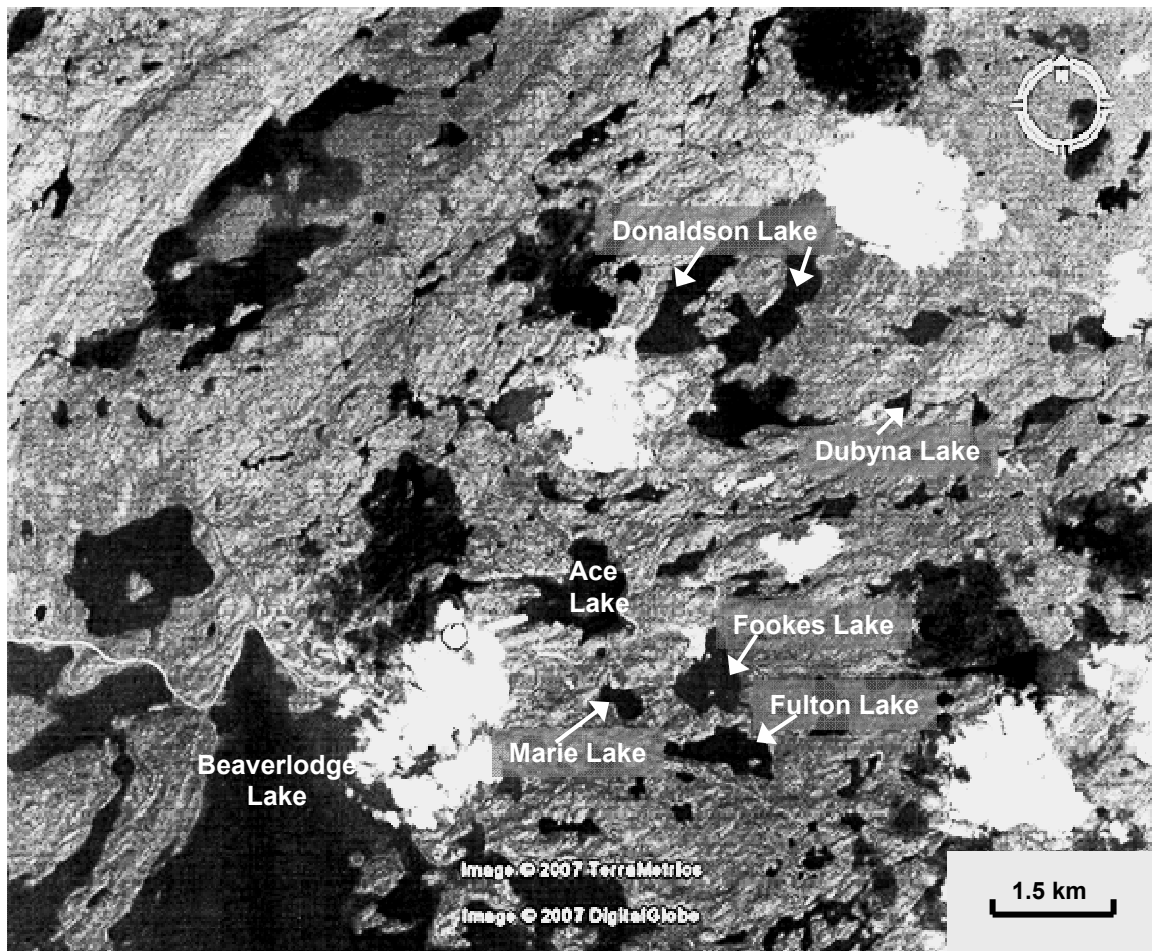
Beaverlodge Mine Area is located in the Canadian Shield just north of the Athabasca Sand Basin (Fig. 2-1, Fig. 2-2). The local topography consists of many ridges and valleys and lakes are often cut-off from the main drainage flows of the surrounding basins. Other elements such as iron, calcium, copper, lead, vanadium, selenium, cobalt, and nickel are often associated with these deposits as secondary elements (Swanson 1982). Uranium mining in the Beaverlodge area was conducted by Eldorado Nuclear Limited from 1952-1982 (Cannorth 2005). Both open-pit and underground mining methods were chosen for uranium extraction.



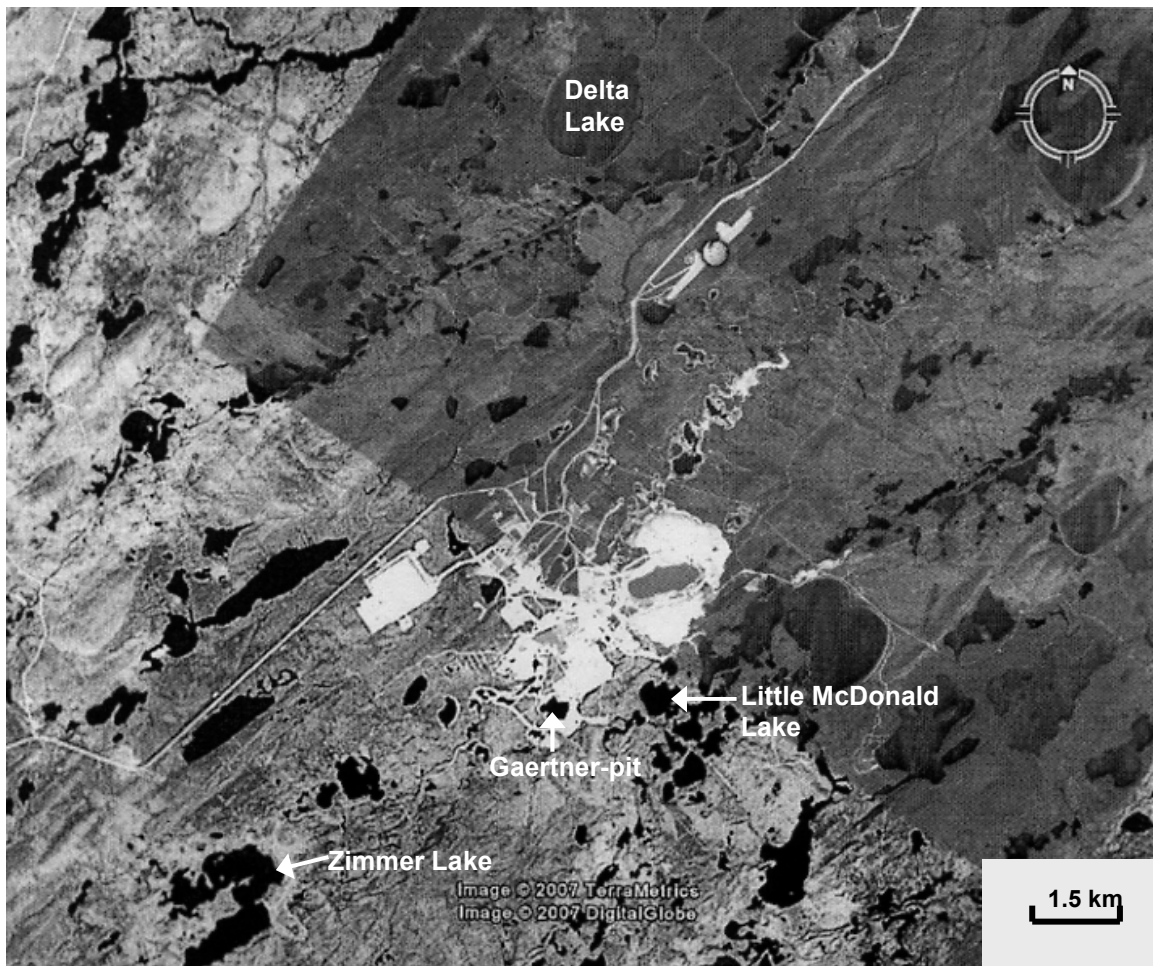
**Figure 2-1.** Mine site locations in northern Saskatchewan.

**Table 2-1.** Summary of the 18 study lakes and 7 study pits and their modes of exposure to potential contaminants. The sampling period is included for each lake. The total number of aquatic samples collected from all lakes and pits was 57.

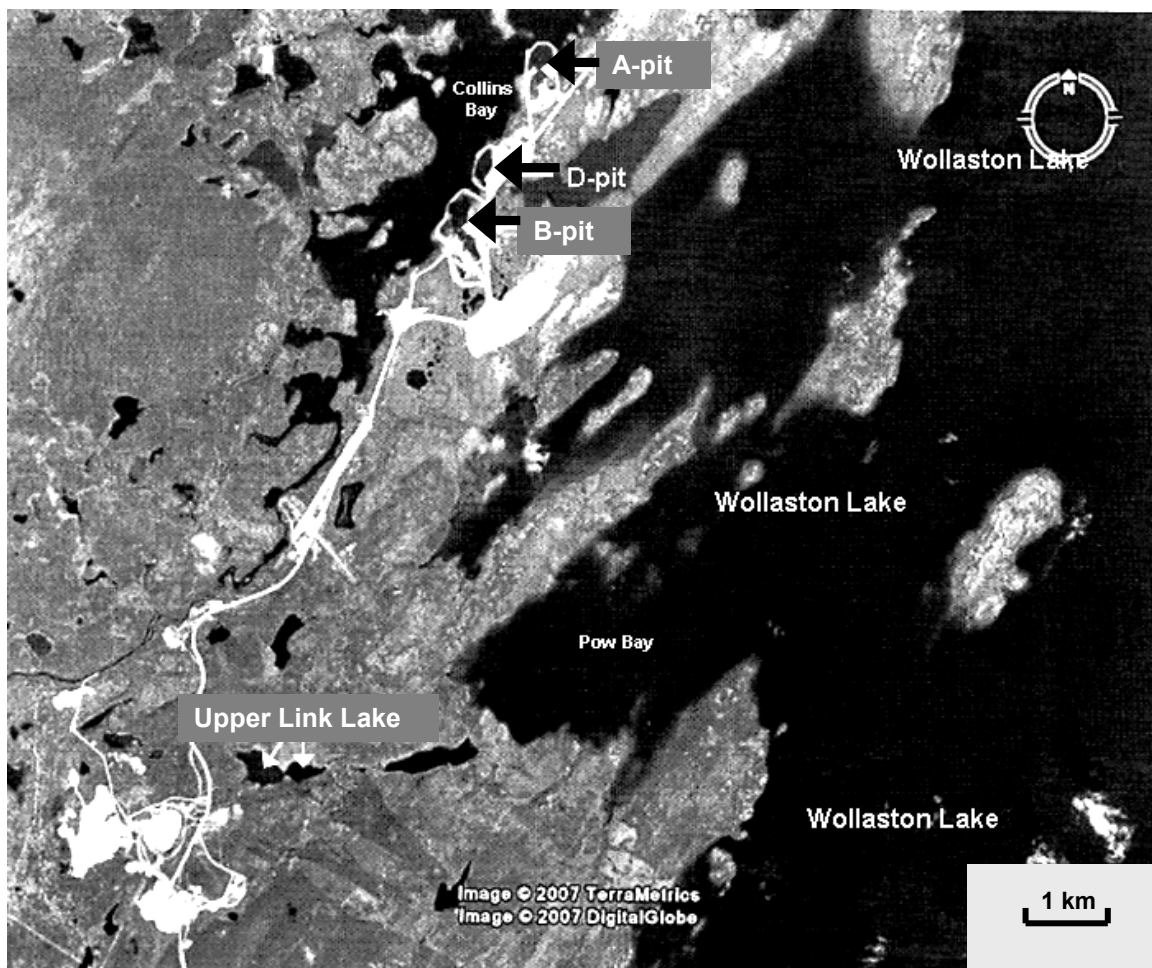
Area	Flooded Pits	Exposed to Treated Effluent	Exposed to Mine Runoff	Miscellaneous Contamination	Reference Lakes
<b>Beaverlodge (Uranium City)</b>			<b>Beaverlodge Lake:</b> Sampled July 2005	<b>Ace Lake:</b> sampled July 2004, 2005	<b>Fredette Lake:</b> sampled July 2004, 2005
<b>Cluff Lake</b>	<b>D-pit:</b> flooded 1983 sampled August 2003, July 2004, August 2005 <b>DJX-pit:</b> flooded 2005 sampled Sept. 2004	<b>Island Lake:</b> sampled August 2003, July 2004, August 2005	<b>Dubyna Lake:</b> sampled July 2003, 2004, 2005 <b>Cluff Lake</b> – minimal exposure makes this a reference lake: sampled August 2003, July 2004, August 2005	<b>Fookes Lake:</b> sampled July 2003, 2004, 2005	<b>Fulton Lake:</b> sampled July 2003, 2004 <b>First Lake:</b> sampled August 2003, July 2004, August 2005 <b>Cluff Lake</b> – minimal exposure makes this a reference lake: sampled August 2003, July 2004, August 2005
<b>Key Lake</b>	<b>Gaertner-pit:</b> sampled August 2005	<b>Delta Lake:</b> sampled July 2003, August 2005	<b>Little McDonald Lake:</b> sampled July 2003, August 2005		<b>Zimmer Lake:</b> sampled July 2003, August 2005
<b>McClean Lake</b>	<b>Sue-C-pit:</b> flooded 2002 sampled July 2003, August 2004	<b>Vulture Lake:</b> sampled August 2004, June 2005 <b>McClean Lake:</b> sampled August 2004, June 2005			<b>Indigo Lake:</b> sampled July 2003, August 2004, June 2005
<b>Rabbit Lake</b>	<b>A-pit:</b> flooded 1997 sampled August 2003, 2004 <b>B-pit:</b> flooded 1992 sampled August 2003, 2004, July 2005 <b>D-pit:</b> flooded 1997 sampled August 2003, 2004, July 2005		<b>Upper Link Lake:</b> sampled August 2003, 2004, July 2005	<b>Wollaston Lake:</b> Receives effluent, mine runoff, and pit-water leakage, but is considered a reference lake due to volume dilution sampled August 2003, 2004, July 2005	



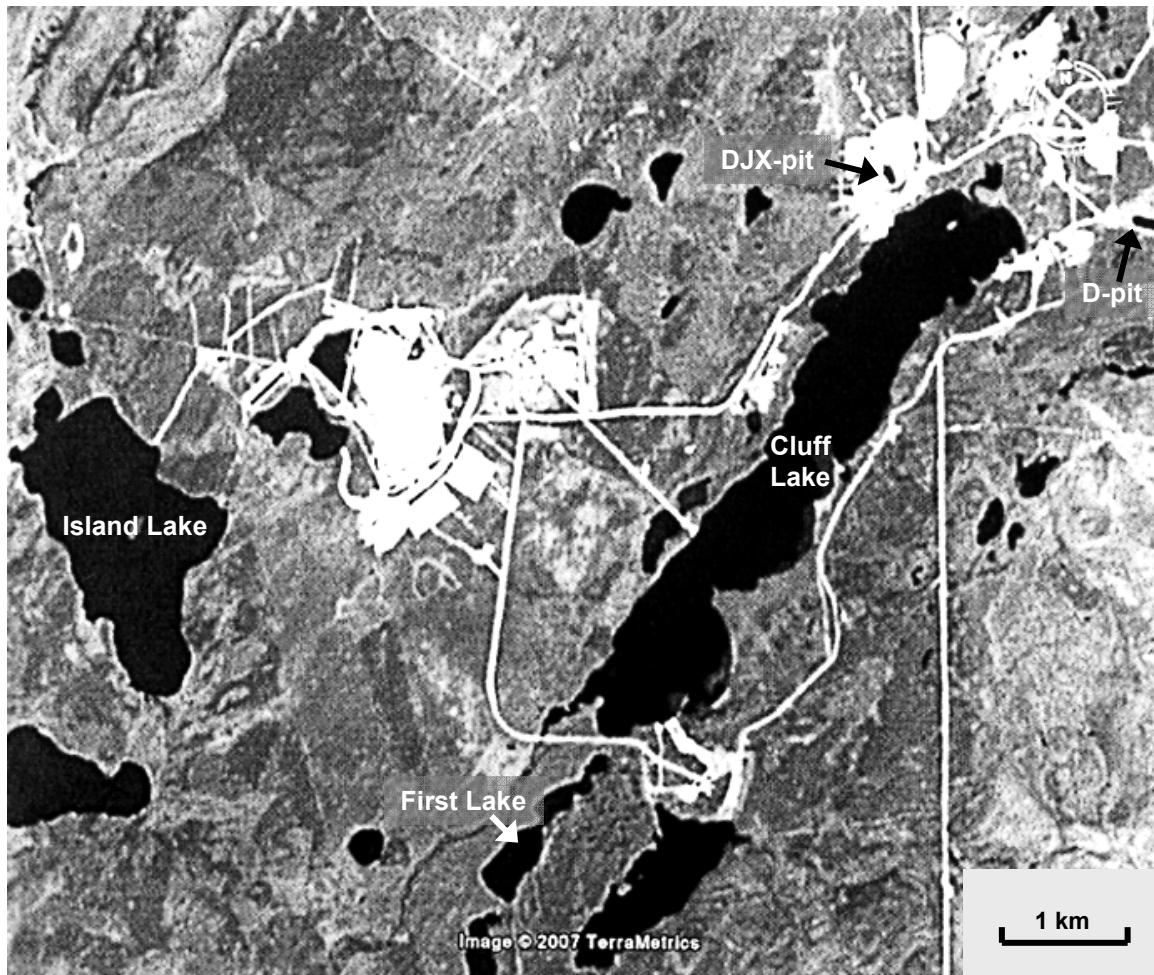
**Figure 2-2.** Study lakes sampled within the Beaverlodge area. White areas are clouds.



**Figure 2-3.** Study lakes at the Key Lake Mine Site.

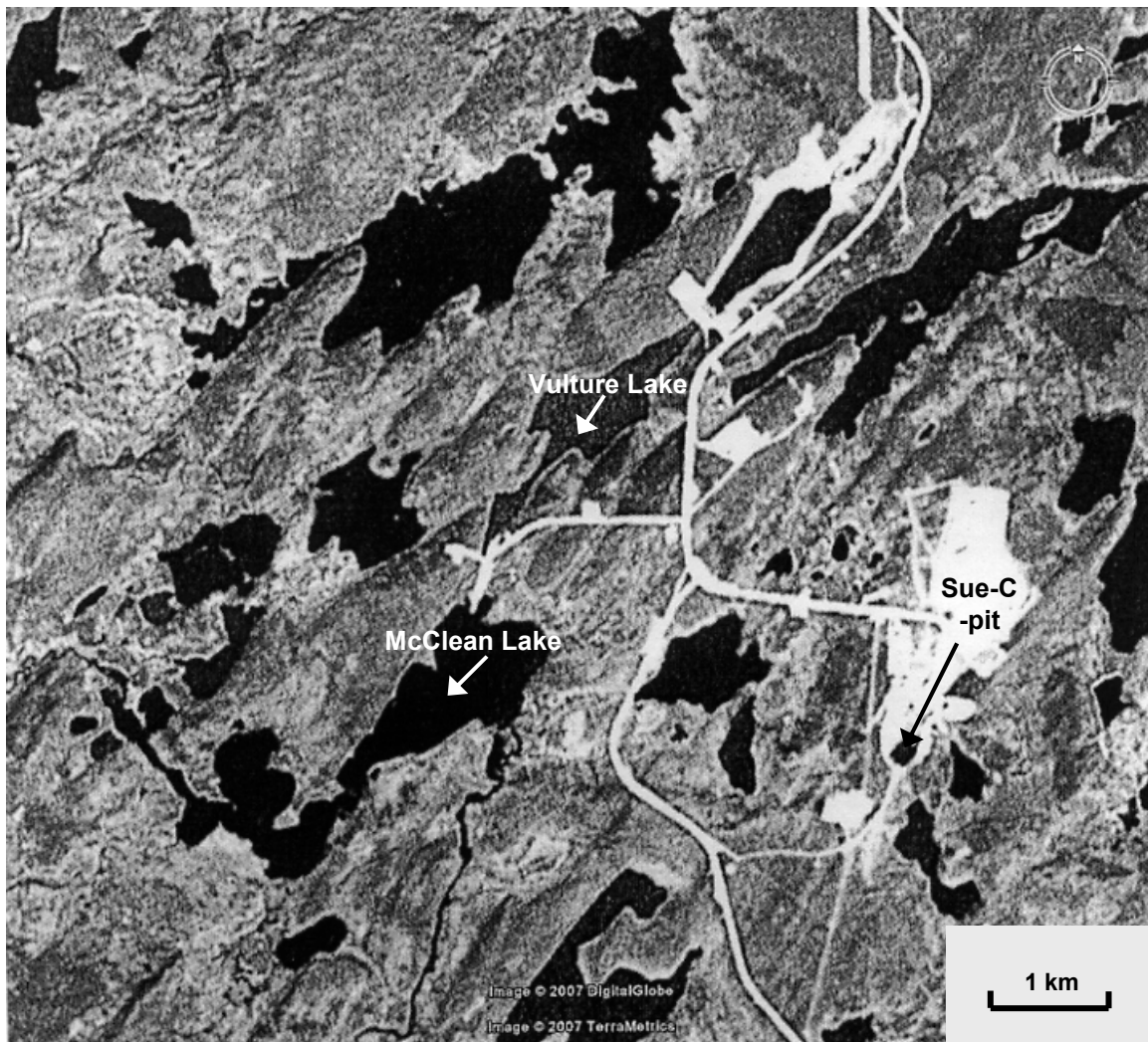


**Figure 2-4.** Study at Rabbit Lake Mine Site.



**Figure 2-5.** Study lakes at Cluff Lake Mine Site.





**Figure 2-6.** Study lakes at McClean Lake Mine Site. Indigo Lake could not be shown within the scale of this map.

### **2.2.1.1 The Fulton Creek Watershed**

The Fulton Creek Watershed contains Fulton Lake, Fookes Lake, Marie Lake, Meadow Lake, and Greer Lake. Milling operations commenced in 1953, with ore coming mainly from nearby underground shafts. Tailings from the milling process, containing high concentrations of total dissolved solids (TDS), uranium, and radium were originally deposited directly into Minewater Lake, located just southeast of the Mill. In 1954, tailings were placed in the larger Marie Lake in an attempt to promote greater settling of contaminants out of the water column. Unfortunately, settling was poor and there was evidence of tailings migration into downstream Greer Lake. As a result, the tailings were again re-routed in 1957 to Fookes Lakes, located immediately upstream of Marie Lake. Tailings continued to be deposited at the northwest shore of Fookes Lake until shut-down in 1982 (Swanson 1982).

The watershed surrounding the tailings management area is  $14.1 \text{ km}^2$ . Marie, Fookes, Fulton, and Greer lakes make up about 13% of this watershed (Eldorado 1983). Weirs were constructed between Fookes and Marie lakes, between Marie and Meadow lakes, and between Meadow and Greer lakes. The purpose of the weirs were to control flows and to also help maintain adequate water cover over the tailings beach area (created by prolonged tailings deposition at Fookes Lake) to increase containment of the tailings (MacLaren 1987). During periods of drought, when water did not cover the tailings beach, runoff coming in contact with the tailings beach area would carry additional uranium, radium, and TDS to Fookes Lake (Senes 1983). Tailings migrated from Fookes to Marie and other downstream lakes via connecting creeks. Tailings spills from malfunctioning sections of the tailings pipeline occupied an area of  $4 \times 10^4 \text{ m}^2$  during operations. Runoff coming in contact with these spills carried additional tailings contamination into the tailing management area (Senes 1983).

Historically, Fookes Lake and Marie Lake were part of this tailings management area, thus both of these lakes are included in this study. Fookes Lake was chosen as our study lake because it is one of the largest lakes in the



watershed and had been directly exposed to tailings for the longest period of time during milling operations. Fookes Lake presents a scenario where we can study biodiversity and ecosystem function in systems that were impacted more than two decades ago and are considered recovering. Since decommissioning, the main source of water to Fookes Lake and the other tailings-receiving lakes is freshwater from Fulton Lake, located upstream of Fookes Lake (Fig. 2-1). However, the tailings beach area is likely a source of continued exposure. Fulton Lake, being upstream from Fookes Lake, was chosen as a reference lake for this area. Fulton Lake is considered a non-impacted lake and the water quality after decommissioning was very close to background levels (SENES Consultants 1983). Current water quality for Fookes, Marie, and Fulton can be found in Table A1-1.

#### **2.2.1.2 The Ace Creek Watershed**

The Ace Creek Watershed occupies an area of approximately 153.6 km<sup>2</sup>. Much of the underground and milling operations were carried out in this watershed. Mining infrastructure included the Ace, Verna, and Fay underground shafts, a waste rock dump site, various water and tailings pipelines, and a sewage treatment lagoon.

Ace Lake and Beaverlodge Lake have been included as study lakes due to their proximity to the former mining areas. Ace lake is located upstream of the mining and milling operations. The Ace underground shaft extended under Ace Lake and Upper Ace Creek brought potentially contaminated water from the Dubyna Lake mining area. Therefore, Ace Lake's water quality was monitored throughout the Eldorado operations. According to the 1983 reports from Eldorado, the water quality of Ace Lake was close to background levels during operations.

Beaverlodge Lake has been impacted due to its downstream location of Ace Creek. Beaverlodge Lake received contaminated outflow from milling operations from 1953-1982. Beaverlodge also received contaminated outflow from Tailings Creek, which flows out of Greer Lake, located within the Fulton

Creek Watershed (Swanson 1982). Increases in TDS,  $U^{+3}$ , and  $Ra-226^{+2}$  were and still are detectable in Beaverlodge Lake near the sources of these inflows.

In addition to Fulton Lake, Fredette Lake was chosen as another reference system. Fredette Lake is also far removed from the former mining and milling areas and supplies the drinking water for Uranium city.

#### **2.2.1.3 Dubyna Lake**

The Dubyna Lake mining area started production in 1979 and consisted of both open-pit and underground mining. Waste rock from the site was deposited near Dubyna Lake, where runoff through the waste rock pile flowed directly into Dubyna Lake. After decommissioning in 1981, the Dubyna mining area continued to release groundwater into Dubyna Lake that contained elevated levels of uranium. Presently, Dubyna Lake still contains uranium levels that are above the target objectives (0.25 mg/L) established by the Atomic Energy Control Board in 1982 (Senes 1983). Dubyna Lake also releases water into Ace Lake via Dubyna Creek, which joins Upper Ace Creek and flows into Ace Lake. Thus, Ace Lake may have been exposed to water containing elevated levels of uranium from Dubyna Lake.

#### **2.2.2 Key Lake Mine Site**

Key Lake Mine Site is located in the south-eastern section of the Athabasca Sand Basin (Fig. 2-1). The Athabasca Basin covers an area of approximately 100,000 km<sup>2</sup>, which is about one third of the Precambrian Shield region in Saskatchewan. The uranium deposits are present in a 20 m – 40 m thick region between the Precambrian Shield and overlying sandstone, known as the regolith region.

Study lakes within the Key Lake area are Delta Lake, Little McDonald Lake, and Zimmer Lake (reference lake) along with one flooded pit-lake, Gaertner Pit (Fig. 2-3). These lakes were chosen based on their exposure to different mining activities (Table 2-1).

#### **2.2.2.1 Delta Lake and Little McDonald Lake**

Delta Lake is located in the David Creek watershed approximately 5 km north of the Key Lake mining and milling area. Delta Lake receives treated effluent from Wolf Lake. Wolf Lake is the first effluent-receiving lake for the area. The treated mill effluent that is discharged into Wolf Lake flows downstream through the following water systems: Wolf Creek, Fox Lake, Yak Creek, David Creek, Delta Lake and the Wheeler River. Although Delta Lake is far downstream from Wolf Lake, there is still evidence of elevated levels of selenium (Cameco 2004). In addition, the concentrations of major ions have increased since 2000, leading to elevated water hardness (Cameco 2004).

Little McDonald Lake is not impacted by treated mill-effluent, but by groundwater generated by the dewatering wells from both Gaertner and Deilmann pits. These dewatering wells control the outflow of contaminated water from these two open pits. Water from these wells is discharged into nearby Horsefly Lake, which flows into Little McDonald Lake. The primary concern with water quality is the elevated concentration of nickel from the dewatering system, which peaked in 1995. In response, a water treatment plant was constructed in 1995 to remove contaminants from the Gaertner and Deilmann dewatering systems. As a result, nickel levels of water flowing into Horsefly Lake had decreased by 90% by 2004 (Cameco 2004). Also, the amount of dewatering water flowing into Horsefly Lake has significantly decreased since 2000 because dewatering water was diverted to Gaertner pit. Decreased contamination of dewatering water and reduced flows to Horsefly Lake has had less impact on Little McDonald Lake in recent years. Little McDonald Lake provides a setting to study the effects of a marginally exposed lake on biodiversity and ecosystem function.

#### **2.2.2.2 Gaertner Pit**

Gaertner pit (an open pit mine) became depleted of viable uranium ore in 1987. In 2000, the commencement of ore processing from McArthur River mine site led to a change in the dewatering system and Gaertner pit was flooded with

water. This created a unique aquatic system that will be referred to as a flooded pit-lake throughout this thesis. As a result of flooding, Cameco Corporation reported increases in calcium, sulfate, nickel, zinc, cobalt, and arsenic in Gaertner Pit in 2004. Hence, this aquatic system provides a setting to study the effects of heavy contamination on biodiversity and ecosystem function in a recently flooded pit.

### **2.2.3 Rabbit Lake Mine Site**

Rabbit lake mine site is located along the western shore of Wollaston Lake. Milling operations at Rabbit Lake began in 1975 and still continue today; both open-pit and underground mining occur at Rabbit Lake. Study lakes from this area include Wollaston Lake, Upper Link Lake, A-pit, B-pit, and D-pit (Fig. 2-4).

#### **2.2.3.1 Wollaston Lake**

Wollaston Lake is 113 km long and 40 km wide, has an area of 2,681 km<sup>2</sup>, and a maximum depth of over 100 m. There are numerous bays, streams, and creeks associated with Wollaston that are in close proximity to the mining activities, making them subject to exposure in various ways. For instance, treated effluent is released into Horseshoe Creek, which drains into Hidden Bay. In 2005, the specific conductivity of the treated effluent was 2018  $\mu\text{S cm}^{-1}$ . However, the specific conductivity in Hidden Bay was only 38  $\mu\text{S cm}^{-1}$  (similar to background conductivity of Wollaston Lake), thus the volume of effluent entering Hidden Bay is insufficient to significantly raise conductivity beyond background levels (Golder 2005).

Pow Bay, located approximately 3 km east of the milling area (Fig. 2-4), is another area of Wollaston Lake that is exposed to mining activity. Pow Bay receives surface run-off from the mine area via the Rabbit Creek drainage system. This runoff enters Upper Link Lake, flows east into Lower Link Lake and discharges into Pow Bay. Until the late 1990's, untreated runoff came from ore stockpiles, the milling area, and dewatering wells from the Rabbit Lake pit (1975-1977 only). Presently, such flows are collected in drainage ditches then pumped

to the mill for treatment before release to the environment. The Rabbit Creek system also received treated effluent between 1975 and 1977. Since 1977, effluent has been released into the Horseshoe Creek drainage system.

Upper and Lower Link lakes show elevated levels of TDS, iron, and radium, however downstream Pow Bay does not have any of these parameters above background levels (Golder 2005). Collins Bay (Fig. 2.4) does not receive any mine runoff or treated effluent. However, A-pit, B-pit, and D-pit are all located along the eastern shore of Collins Bay and are isolated from Collins Bay by specially constructed Dams (coffer dams). Due to the proximity of these pits, Collins Bay has been marginally exposed to surface runoff and possible leaking of the pits into the Bay, however, water quality has remained at background levels. Although exposure is evident, Wollaston Lake is considered a reference system, especially in the open water regions where samples for this study were collected.

#### **2.2.3.2 Upper Link Lake**

Upper Link also received untreated runoff from the mine site area and from ore stockpiles from 1975 until the late 1990's. Presently, all runoff is collected and treated at the mill before it is released to Upper Link Lake. Golder Associates Ltd. (2005) reported that Upper Link Lake showed elevated levels of TDS, iron, and radium. Our water quality data supports these observations, showing elevated levels of TDS and hardness relative to other study lakes (Table A1-1).

#### **2.2.3.3 A-pit, B-pit, and D-pit**

Pit-lakes commonly have low pH, high metal concentrations, and high TDS (Levy et al. 1997). B-pit (Fig. 2-4), which was flooded with water in the winter of 1991/1992 from adjacent Collins Bay, has showed elevated concentrations of nickel and arsenic. Until 1998, external loading of nickel was attributed to runoff from an adjacent road. After demolition of the road in 1998, external loading of nickel was eliminated and concentrations of both nickel and arsenic have declined steadily. Our water quality data suggests that nickel and

arsenic concentrations in surface water remain above Saskatchewan Surface Water Quality Objectives (SSWQO) and are greater relative to the other lakes in our data set.

The A-pit and D-pit ore bodies were discovered in 1971. Both A-pit and D-pit ore deposits were completely submerged beneath Collins Bay along the east shoreline (Fig. 2-4). In order for open-pit mining to be possible, a dyke system was constructed to isolate both pit areas from Collins Bay. After dyke construction, the zones were dewatered and the sediments were dried to allow for mining to begin. D-pit was constructed in 1995 and was depleted of ore by 1996, while A-pit was constructed in 1996, and was mined out by 1997. Once mining was completed, both pits were backfilled with clean waste rock, topped with 4m of sand and till, and then flooded with water and sediment from Collins Bay (completed September 1997). A-pit, B-pit, and D-pit all differ in water quality from one another and were flooded at different times. These pits provide another opportunity to compare biodiversity and ecosystem function.

#### **2.2.4 Cluff Lake Mine Site**

Cluff Lake Mine Site is located approximately 75 km south of the southwestern shore of Lake Athabasca in the Athabasca Sand Basin. Mining and milling began in 1980 and the operation has produced over 28 million kg of uranium ( $U_3O_8$ ) from open pits and underground mines. The Island Creek watershed and the Cluff Creek watershed were exposed to mining activities. Cluff Lake Mine Site is now in its final stages of decommissioning; all mining has stopped and the environment is being restored to a natural state. Study lakes from this area include Cluff Lake (reference), First Lake (reference), Island Lake, D-pit, and DJX-pit (Fig. 2-5).

##### **2.2.4.1 Island Lake**

Treated effluent from the Cluff Lake Mill was discharged into the Island Creek watershed. The release point of the effluent stream starts at Snake Creek, which drains into Island Lake, making Island Lake the first effluent-receiving lake in the area. Island lake is quite shallow (mean depth = 1.5m, max. depth =

2.2m), but is the second largest lake in the mining area with a surface area of 181 ha. The most prominent impact from effluent exposure has been a 28 fold increase in specific conductivity (salinity) since the pre-operational period (Cogema 2000).

#### **2.2.4.2 D-pit and DJX-pit**

D-pit is located within the Cluff Creek watershed (Fig. 2-5) along with the adjacent Boulder Creek. Although Boulder Creek flows into Cluff Lake, D-pit is isolated from the watershed and no impact on Boulder Creek or Cluff Lake has been observed. D-pit was mined from 1979-1981 and contained the highest grade of U among all viable deposits. D-pit was flooded in 1983 due to an overflowing of Boulder Creek and the pit has remained flooded since 1983. DJX-pit was mined from 1994-1997. DJX Pit was sampled once in 2004.

#### **2.2.4.3 Cluff Lake and First Lake**

Cluff Lake belongs to the Cluff Creek watershed area, which is completely separate from the Island Lake watershed. Cluff Lake is the largest lake in the mining area with a surface area of 341 ha, a maximum depth of 52 m, and a mean depth of 19.9 m. Cluff Lake receives no treated effluent discharge but nearby D-pit, DJX, DJN, and Claude pit have the potential to affect Cluff Lake through ground water seepage and runoff.

The pre-operational Environmental Impact Statement (EIS) predicted marginal changes in water quality and biota in the Cluff Creek watershed. During operations water quality and biotic impacts within the Cluff Creek drainage area, including Cluff Lake, are considered negligible (Cogema 2000).

#### **2.2.5 McClean Lake Mine Site**

The McClean Lake operation is located 15 km west of Wollaston Lake (Fig. 2-1). Operations began in the mid-1990's and the site is still in production. All mining facilities are located within the Collins Creek Watershed and the adjacent Moffat Creek Watershed. Study lakes include Indigo Lake (reference), McClean Lake, Vulture Lake, and Sue-C pit (Fig. 2-6).

#### **2.2.5.1 McClean Lake, Vulture Lake, and Indigo Lake**

Vulture Lake and McClean Lake are part of the Sink/Vulture Treated Effluent Management System. Treated effluent from the mill is released into Sink Lake, which flows into Vulture Lake, then McClean Lake. Indigo lake is far removed from the mining area and receives no exposure to mining activities and is considered a reference system for this area.

#### **2.2.5.2 Sue-C pit**

Mining of Sue-C pit began in 1997 and ended February of 2002. The pit was allowed to start flooding naturally in 2002. Sue-C Pit represents another recently flooded pit-lake for this study.

### **2.3 Statistical Methods**

#### **2.3.1 Classification of Lakes**

Appendix 1 contains a data matrix of water quality variables (columns) by lakes (rows). This data matrix consists of water quality data provided by Areva Resources Canada Inc. and Cameco Corporation. Quality Assurance (QA) and Quality Control (QC) procedures are routinely carried out by Cameco and AREVA to ensure that the water quality data is credible. Water quality data was selected for the month in which I sampled a particular lake or pit. For example, if I collected a sample from a lake or pit, then the June 2004 water quality data (from industry) for that particular lake or pit was used for analysis. Hierarchical clustering is often used to identify groups of samples when multiple variables are being analyzed (McCune and Grace 2002). Such methods provide hierarchical dendrograms that create larger groups from smaller sub-groups until all variation is accounted for in all variables. One such method is Ward's Method of classification (McCune and Grace 2002). It is based on a minimum variance criterion in which new groups are based on a minimum increase in the distance sum of squares over those of its two constituent groups.

Cluster analysis was preceded by two data manipulations. First, data were Z-scored to place equal weighting on all variables in the analysis. This was



necessary since many variables were measured in different units (e.g. pH, mg L<sup>-1</sup>, µg L<sup>-1</sup>, °C) and size scales. Second, the calculation of the distance matrix for all possible pairs of lake and pit samples was completed prior to classification by Ward's Method. Ward's Method was applied using PC-ORD (McCune and Mefford 1999), a multivariate statistics package designed for ecological applications. This method of cluster analysis does not alter the positioning (distance) of previously established groups as new groups are formed. The resulting dendrogram does not suffer from chaining in which samples are added to existing groups one at a time. Chaining leads to poorly separated groups and the resulting dendrograms are often difficult to interpret (McCune and Grace 2002).

Determining the optimum number of groups to use is essential when interpreting dendrograms. A method known as indicator analysis was used to determine this optimum number of groups (McCune and Grace 2002). Indicator analysis looks for the water quality variables that are representative of each group. The method provides a table of p-values showing which variables are significant indicators of different groups. Indicator analysis was run at different group levels (the range for this study was 10 lake groups down to 2 lake groups) and p-values were determined for all variables at the various group levels. The average p-value and the number of significant indicators (variables with p-values ≤ 0.05) were then calculated for each group-level. A low average p-value and a high number of significant indicators represent an optimum number of lake groups (McCune and Grace 2002).

### **2.3.2 Testing for Significant Differences among Lake Groups**

Once the optimum number of groups was determined, the significance of the difference among groups is determined. Multi-Response Permutation Procedures (MRPP) was used to accomplish this task. MRPP works well with multivariate ecological (McCune and Grace 2002) data because it avoids the assumptions of normality and homogeneity that are required in other tests for

significance, such as Multivariate Analysis of Variance (MANOVA). MRPP produces three statistics:

- i. Test statistic,  $T$ . High negative values indicate greater separation between groups (i.e. groups are different from one another).
- ii. Chance corrected within-group agreement,  $A$ . This describes the within-group homogeneity, compared to the random expectation. When  $A = 1$  (highest possible value), all entities within groups are identical. The closer  $A$  is to 1, the more agreement there is within the group, and therefore, the more likely the group is different from other groups.
- iii. Statistical significance, p-values. This shows the probability of obtaining a difference between groups by chance. For example, when  $p \leq 0.05$ , or lower, there is a 5% chance, or less, of observing the same result by chance.

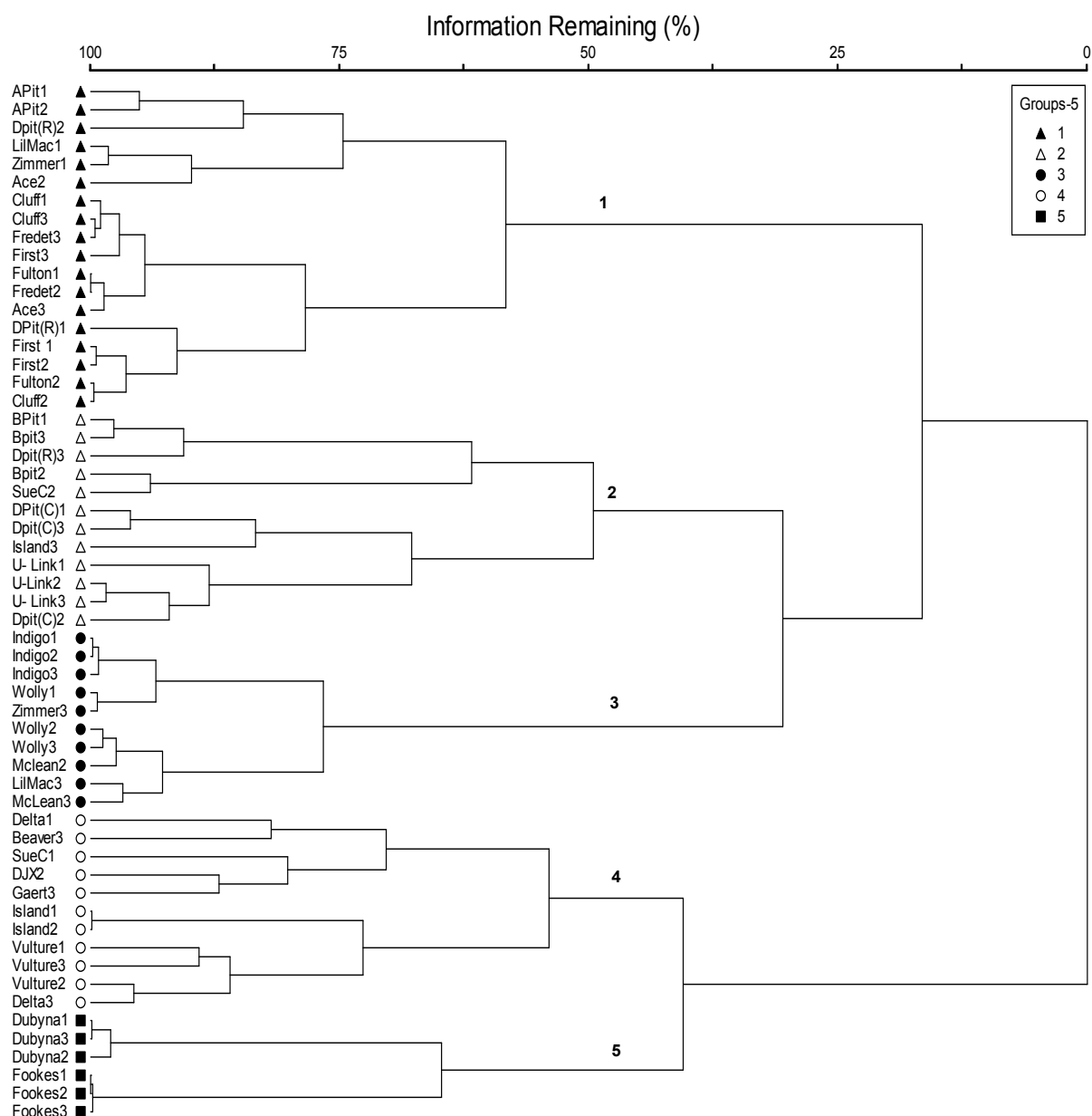
## **2.4 Results**

The cluster dendrogram, created by Ward's Method (Fig. 2-7), shows how the sample lakes group together. The number of groups decreases and the size of the groups increases as more information, provided by the standardized water quality matrix, is taken into consideration by the analysis. The lakes contained in each group are associated with their representative symbols (Fig. 2-7)

The lowest average p-values, resulting from indicator analysis, exist at the 3 and 5 group-level (Table 2-2 and Fig. 2-8 a). The number of significant indicator variables is identical for group levels 3, 5 and 7 (Table 2-2 and Fig. 2-8 b) making these group-levels the most appropriate relative to the other group levels. Further interpretation led to the determination that the 5 group level was more appropriate than the 3 group level (see discussion).

The five lake groups are significantly different and all groups are significantly different from each other (MRPP,  $p \leq 0.05$ , Table 2-3). However, the water quality variables that define each group have yet to be determined. These defining variables are determined by indicator analysis (Table 2-4). Indicator analysis did not reveal any significant indicator variables for lake groups 1 and 3.

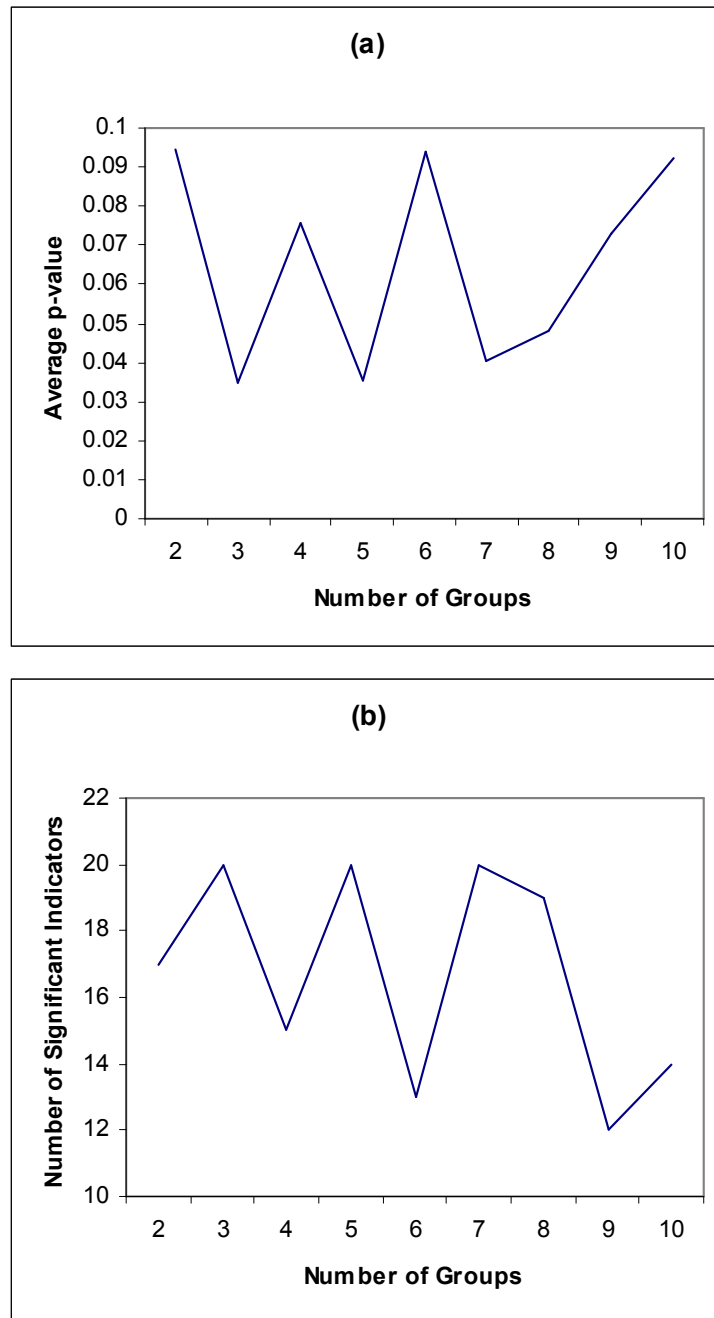
The topology of the cluster dendrogram (Fig. 2-7) suggests that groups 2 and 3 are more similar to one another than to group 1. Therefore, to appropriately determine differences between groups 1 and 3, independent analyses of group 1 versus group 2 and group 1 versus group 3 were necessary. The variables pH and bicarbonate were indicators for group 1 in both analyses (Table 2-5). Aluminum and iron were indicator variables for groups 2 and 3 in both analyses. These explain the lack of indicators for groups 1 and 3 in the primary analysis. However, other indicators were also found for group 1 and group 2 in the two analyses, respectively, and explain why 5 groups were identified in the primary analysis despite the lack of indicators for all groups.



**Figure 2-7.** Classification of aquatic samples by Ward's Method, using 24 water quality variables measured for each lake and pit. This dendrogram shows the lake groups at the five-group level identified by different symbols. From left to right, the number of groups decreases and the number of aquatic systems per group increase as the percent information remaining in the distance matrix approaches zero. Lakes denoted by a 1 (e.g. A-PIT 1) were sampled in 2003, lakes denoted by a 2 were sampled in 2004, while lakes denoted by a 3 were sampled in 2005.

**Table 2-2.** Indicator analysis of aquatic systems at various group levels (2 – 10). Significant p-values are in bold text. The groups levels were created by Ward's Method of cluster analysis. The average p-values and the number of significant indicators ( $p \leq 0.05$ ) are calculated for each group-level at the bottom of the table.

Variables	GROUP LEVELS AND ASSOCIATED P-VALUES FROM INDICATOR ANALYSIS									
	2	3	4	5	6	7	8	9	10	
1 Temp (°C)	0.188	<b>0.008</b>	<b>0.011</b>	0.091	0.289	0.217	0.386	0.118	0.287	
2 TDS (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.016</b>	<b>0.006</b>	<b>0.022</b>	<b>0.003</b>	<b>0.009</b>	0.072	0.076	
3 pH	0.888	0.113	0.096	<b>0.027</b>	0.065	<b>0.051</b>	0.081	0.233	<b>0.03</b>	
4 As (ug L <sup>-1</sup> )	0.239	<b>0.036</b>	<b>0.001</b>	<b>0.008</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.005</b>	<b>0.003</b>	
5 Al (mg L <sup>-1</sup> )	0.372	<b>0.017</b>	<b>0.004</b>	<b>0.012</b>	0.085	0.153	0.137	<b>0.011</b>	<b>0.017</b>	
6 Ba (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.002</b>	<b>0.002</b>	<b>0.004</b>	<b>0.017</b>	<b>0.016</b>	0.074	<b>0.027</b>	
7 B (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.004</b>	0.079	<b>0.025</b>	0.131	0.297	0.327	0.269	0.34	
8 Ca (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.002</b>	<b>0.004</b>	<b>0.008</b>	<b>0.001</b>	<b>0.001</b>	<b>0.013</b>	<b>0.013</b>	
9 Cl (mg L <sup>-1</sup> )	<b>0.023</b>	<b>0.013</b>	0.097	<b>0.045</b>	0.153	<b>0.013</b>	<b>0.017</b>	0.131	0.214	
10 Cu (mg L <sup>-1</sup> )	0.187	0.358	0.698	0.301	0.517	<b>0.044</b>	<b>0.027</b>	0.119	0.199	
11 Fe (mg L <sup>-1</sup> )	<b>0.048</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.003</b>	
12 HCO <sub>3</sub> (mg L <sup>-1</sup> )	0.185	0.106	<b>0.054</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.005</b>	<b>0.003</b>	
13 K (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.003</b>	<b>0.001</b>	<b>0.004</b>	<b>0.001</b>	<b>0.001</b>	<b>0.008</b>	<b>0.004</b>	
14 Mg (mg L <sup>-1</sup> )	<b>0.023</b>	<b>0.028</b>	0.093	<b>0.023</b>	0.101	<b>0.013</b>	<b>0.014</b>	0.095	0.112	
15 Mn (mg L <sup>-1</sup> )	<b>0.011</b>	<b>0.022</b>	0.174	<b>0.054</b>	0.181	<b>0.01</b>	<b>0.008</b>	0.087	0.197	
16 Na (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	0.084	0.068	0.17	<b>0.013</b>	<b>0.017</b>	0.12	0.211	
17 Ni (mg L <sup>-1</sup> )	0.067	0.089	0.224	0.121	0.264	<b>0.004</b>	<b>0.006</b>	0.084	0.12	
18 Mo (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.002</b>	<b>0.012</b>	<b>0.003</b>	<b>0.008</b>	<b>0.001</b>	<b>0.003</b>	<b>0.024</b>	<b>0.026</b>	
19 Ra226(Bq L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.012</b>	<b>0.001</b>	<b>0.003</b>	<b>0.007</b>	<b>0.006</b>	<b>0.044</b>	<b>0.003</b>	
20 Se (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.003</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.001</b>	<b>0.013</b>	<b>0.007</b>	
21 SO <sub>4</sub> (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.008</b>	<b>0.004</b>	<b>0.012</b>	<b>0.001</b>	<b>0.001</b>	<b>0.014</b>	<b>0.022</b>	
22 U (ug L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.003</b>	<b>0.014</b>	<b>0.052</b>	0.119	0.086	0.148	0.232	
23 Zn (mg L <sup>-1</sup> )	<b>0.026</b>	<b>0.027</b>	0.123	<b>0.024</b>	0.151	<b>0.001</b>	<b>0.001</b>	<b>0.045</b>	<b>0.045</b>	
24 Hardness (mg L <sup>-1</sup> )	<b>0.001</b>	<b>0.001</b>	<b>0.014</b>	<b>0.005</b>	<b>0.025</b>	<b>0.001</b>	<b>0.001</b>	<b>0.022</b>	<b>0.029</b>	
AVG.	0.095	<b>0.035</b>	0.076	<b>0.035</b>	0.094	0.040	0.048	0.073	0.093	
# Sig. Indicators	17	20	15	20	13	20	19	12	14	



**Figure 2-8.** Determination of an appropriate number of lakes groups. (a) Average p-values for each lake group level, from Table 2-2. The lowest average p-values are at the 3-group-level and the 5-group-level making these two group-levels the most appropriate. (b) The highest number of significant indicator variables ( $p \leq 0.05$  for 20 of 24 variables) also includes group levels 3 and 5.

**Table 2-3.** Separation of lake groups using MRPP. Pair-wise analysis was completed using combinations among the five lake groups. P-values reflect highly significant differences between all groups, as well as between all pair-wise combinations. Highly negative T-values correspond with highly significant p-values. A-values that are closer to 1 represent more within-group homogeneity among the two groups compared, which increases the probability that two groups will be significantly different from one another.

<b>Group #</b>	<b>T</b>	<b>A</b>	<b>p-value</b>
<b>All Groups</b>	-24	0.32	$0.0 \times 10^{-8}$
<b>1 vs. 2</b>	-14	0.21	$3.0 \times 10^{-8}$
<b>1 vs. 3</b>	-14	0.35	$2.1 \times 10^{-7}$
<b>1 vs. 4</b>	-15	0.19	$4.0 \times 10^{-8}$
<b>1 vs. 5</b>	-11	0.26	$1.7 \times 10^{-7}$
<b>2 vs.3</b>	-10	0.22	$1.7 \times 10^{-6}$
<b>2 vs. 4</b>	-9	0.15	$4.2 \times 10^{-6}$
<b>2 vs. 5</b>	-8	0.27	$1.4 \times 10^{-5}$
<b>3 vs. 4</b>	-10	0.17	$6.3 \times 10^{-6}$
<b>3 vs. 5</b>	-8	0.34	$2.5 \times 10^{-5}$
<b>4 vs.5</b>	-6	0.15	$1.0 \times 10^{-4}$

**Table 2-4.** The grouping of lakes and pits with Indicator Analysis showing the lake groups, their constituent lakes and indicator variables. Lakes denoted by a 1 (e.g. A-PIT 1) were sampled in 2003, lakes denoted by a 2 were sampled in 2004, while lakes denoted by a 3 were sampled in 2005.  $P \leq 0.05$  for all significant indicator variables.

Group #	Lakes Included in Group	Significant Indicator Variables
1	<p><b>Flooded Pits:</b> A-Pit 1, A-Pit 2, D-Pit(Rabbit Lake)1 D-Pit(Rabbit Lake)2</p> <p><b>Reference Lakes:</b> Zimmer 1, Cluff 1, Cluff 2, Cluff 3, Fredette 2, Fredette 3, First 1, First 2, First 3, Fulton 1, Fulton 2</p> <p><b>Miscellaneous Contamination:</b> Ace 2, Ace 3</p> <p><b>Exposed To Mine Runoff:</b> Little McDonald 1</p>	NONE
2	<p><b>Flooded Pits:</b> B-Pit 1, B-Pit 2, B-Pit 3, D-Pit (Cluff Lake) 1, D-Pit(Cluff Lake) 2, D-Pit (Cluff Lake) 3, D-Pit(Rabbit Lake)3, Sue-C 2</p> <p><b>Exposed To Treated Effluent:</b> Island 3</p> <p><b>Exposed To Mine Runoff:</b> Upper Link 1, Upper Link 2, Upper Link 3</p>	arsenic ( $\text{As}^{-3}$ ), aluminum ( $\text{Al}^{+3}$ ), iron ( $\text{Fe}^{+2}$ )



(Table 2-4 continued)

Group #	Lakes included in group	Significant Indicator Variables
3	<b>Reference Lakes:</b> Indigo 1, Indigo 2, Indigo 3, Wollaston 1, Wollaston 2, Wollaston 3, Zimmer 3  <b>Exposed To Treated Effluent:</b> McClean 2, McClean 3  <b>Exposed To Mine Runoff:</b> Little McDonald 3	NONE
4	<b>Flooded Pits:</b> Sue-C 1, DJX-Pit 2, Gaertner-Pit 3  <b>Exposed To Mine Runoff:</b> Beaverlodge 3  <b>Exposed To Treated Effluent:</b> Island 1, Island 2, Vulture 1, Vulture 2, Vulture 3, Delta 1, Delta 3	total dissolved solids (TDS), boron ( $B^{+3}$ ), calcium ( $Ca^{+2}$ ), chlorine ( $Cl^{-}$ ), potassium ( $K^{+}$ ), magnesium ( $Mg^{+2}$ ), manganese ( $Mn^{+2}$ ), sodium ( $Na^{+}$ ), molybdenum ( $Mo^{+6}$ ), selenium ( $Se^{-2}$ ), sulfate ( $SO_4^{-2}$ ), zinc ( $Zn^{+2}$ ), hardness
5	<b>Miscellaneous Contamination:</b> Fookes 1, Fookes 2, Fookes 3  <b>Exposed To Mine Runoff:</b> Dubyna 1, Dubyna 2, Dubyna 3	bicarbonate ( $HCO_3^{-}$ ), uranium ( $U^{+3}$ ), barium ( $Ba^{+2}$ ), radium ( $Ra-226^{+2}$ ), pH

**Table 2-5.** Indicator analysis of group 1 versus group 3 and group 1 versus group 2. Indicator variables are shown where  $\alpha \leq 0.10$ . Less significant variables have been accepted in the analysis to explain the separation of group 1 from group 3 and group 1 from group 2 in cluster analysis.

Group 1 vs 3	Lake Included in Group	Indicator Variables
1	<p><b>Flooded Pits:</b> A-Pit 1, A-Pit 2, D-Pit(Rabbit Lake)1 D-Pit(Rabbit Lake)2</p> <p><b>Reference Lakes:</b> Zimmer 1, Cluff 1, Cluff 2, Cluff 3, Fredette 2, Fredette 3, First 1, First 2, First 3, Fulton 1, Fulton 2</p> <p><b>Exposed To Mine Runoff:</b> Little McDonald 1</p> <p><b>Miscellaneous Contamination:</b> Ace 2, Ace 3,</p>	<p>pH, temperature, calcium (<math>\text{Ca}^{+2}</math>), chlorine (<math>\text{Cl}^-</math>), bicarbonate (<math>\text{HCO}_3^-</math>), magnesium (<math>\text{Mg}^{+2}</math>), hardness, uranium (<math>\text{U}^{+3}</math>)</p>
3	<p><b>Reference Lakes:</b> Indigo 1, Indigo 2, Indigo 3, Wollaston 1, Wollaston 2, Wollaston 3, Zimmer 3</p> <p><b>Exposed To Treated Effluent:</b> McClean 2, McClean 3</p> <p><b>Exposed To Mine Runoff:</b> Little McDonald 3</p>	<p>aluminum (<math>\text{Al}^{+3}</math>), iron (<math>\text{Fe}^{+2}</math>)</p>

(Table 2-5 continued)

Group 1 vs 2	Lake included in group	Indicator Variables
<b>1</b>	<p><b>Flooded Pits:</b> A-Pit 1, A-Pit 2, D-Pit(Rabbit Lake)1 D-Pit(Rabbit Lake)2</p> <p><b>Reference Lakes:</b> Zimmer 1, Cluff 1, Cluff 2, Cluff 3, Fredette 2, Fredette 3, First 1, First 2, First 3, Fulton 1, Fulton 2</p> <p><b>Exposed To Mine Runoff:</b> Little McDonald 1</p> <p><b>Miscellaneous Contamination:</b> Ace 2, Ace 3,</p>	pH, bicarbonate ( $\text{HCO}_3^-$ ),
<b>2</b>	<p><b>Flooded Pits:</b> B-Pit 1, B-Pit 2, B-Pit 3, D-Pit (Cluff Lake) 1, D-Pit(Cluff Lake) 2, D-Pit (Cluff Lake) 3, D-Pit(Rabbit Lake)3, Sue-C 2</p> <p><b>Exposed To Treated Effluent:</b> Island 3</p> <p><b>Exposed To Mine Runoff:</b> Upper Link 1, Upper Link 2, Upper Link 3</p>	arsenic ( $\text{As}^{-3}$ ), aluminum ( $\text{Al}^{+2}$ ), copper ( $\text{Cu}^{+2}$ ), iron ( $\text{Fe}^{+2}$ ), manganese ( $\text{Mn}^{+2}$ ), sodium ( $\text{Na}^+$ ), nickel ( $\text{Ni}^{+2}$ ), molybdenum ( $\text{Mo}^{+6}$ ), radium ( $\text{Ra-226}^{+2}$ ), selenium ( $\text{Se}^{-2}$ ), sulfate ( $\text{SO}_4^{-2}$ ), uranium ( $\text{U}^{+3}$ )

## 2.5 Discussion and Conclusions

The objective of this chapter was to classify lakes based on similarities and differences in water quality. Five significantly different lakes groups were established. Ward's cluster analysis identified a number of grouping options, but indicator analysis revealed that five lake groups were the most appropriate. More discussion is required to understand why certain study lakes were grouped together and to provide support for the choice of 5 lake groups over 3 lake groups. A review of the water chemistry data will assist in explaining the classification (Tables A2-1 to A2-6). For example, lake group 4 has a large

number of significant indicator variables (Table 2-4). The concentrations of these variables in group 4 are often several times greater than the concentrations in other lakes (Table A2-4). Concentrations of analytes were approximately 7 fold (TDS) to 4 fold ( $\text{Zn}^{+2}$ ) greater in group 4 lakes compared to the other study lakes (Appendix 2). Concentrations of the indicator variables identified for each lakes group were higher than the concentrations of those same variables in the other lakes groups. This implies that the indicator analysis has identified appropriate variables for each lake group.

Group 4 includes all of the effluent receiving lakes, as well as a few aquatic systems that were not exposed to effluent. The effluent receiving lakes are included due to their elevated concentrations of major ions (i.e.  $\text{Mg}^{+2}$ ,  $\text{Na}^{+}$ ,  $\text{Ca}^{+2}$ ,  $\text{Cl}^{-}$ , and  $\text{SO}_4^{-2}$ ). The non-effluent receiving systems of this group are included due to their elevated concentrations of  $\text{Mo}^{+6}$ ,  $\text{Zn}^{+2}$ ,  $\text{Mn}^{+2}$ , and  $\text{Se}^{-2}$ . Elevated selenium concentration in Beaverlodge Lake ( $2.3 \mu\text{g L}^{-1}$ ) are likely explained by inflowing water from Ace Creek and Tailings Creek. The sampling location for Beaverlodge Lake was near the inflow point of Ace Creek and it is reasonable that contaminants are present in higher concentrations.

Selenium was quite high in the effluent receiving Delta Lake 1 (2003) likely due to its location in the David Creek watershed which receives treated effluent that is known to contain elevated concentrations of Se (Cameco 2004). The selenium concentration in Delta Lake 3 (2005) was identical to the mean selenium concentration of lakes outside group 4, however, the elevated concentrations of major ions and Mo likely resulted in the placement of Delta 3 in group 4. .

The indicator variables for lake group 5 were  $\text{Ra-226}^{+2}$ ,  $\text{U}^{+3}$ , barium ( $\text{Ba}^{+2}$ ), bicarbonate ( $\text{HCO}_3^{-}$ ), and pH (Table 2-4). This lake group consists of Fookes Lake (all three sample years) and Dubyna Lake (all three sample years). As mentioned earlier, Fookes Lake was part of the tailings management area of the Beaverlodge Mining Area. The tailings are a source of radium and uranium, which explains the elevated levels of these elements in Fookes Lake relative to the other lakes. Dubyna lake is also exposed to elevated levels of uranium and

radium in runoff from the nearby waste rock pile created by the Dubyna pit. High levels of bicarbonate would also explain why pH came out as an indicator variable. The mean pH for Fookes and Dubyna together was 8.2 compared to 7.6 for all other lakes. These two lakes are nearly an order of magnitude more basic than the other lakes. High bicarbonate concentrations are associated with pH levels of 8 to 9 where bicarbonate becomes the dominant form of inorganic carbon (Kalff 2002). Barium is a significant indicator of lake group 5 because both lakes were treated with barium chloride in the late 1970's to precipitate radium-226 out of the water column (Cannorth 2005).

Aluminum ( $\text{Al}^{+3}$ ), iron ( $\text{Fe}^{+2}$ ), and arsenic ( $\text{As}^{-3}$ ) were the significant water quality variables for lake group 2. Group 2 consists of flooded mine pits (D-pit-Rabbit, D-pit-Cluff, Sue-C-pit, and B-pit) and two lakes (Island and Upper Link). Flooded mine pits are often associated with elevated levels of metals (Nyogi et al. 2001), especially common elements such as  $\text{Al}^{+3}$  and  $\text{Fe}^{+2}$ . Metals of concern are often site-specific (Pyle et al. 2001), implying that As is likely a naturally occurring element in the Athabasca Sand Basin. The major contributor of metals to these pits is the leaching of contaminants out of the pit walls upon flooding. Weathering of the pit walls, before and after flooding, contributes further to metals in solution. Flooded pits in lake group 2 have  $\text{Al}^{+3}$ ,  $\text{As}^{-3}$ , and  $\text{Fe}^{+2}$  concentrations that are 3.8, 11.9, and 6.2 times greater than those in all other study lakes, respectively. It seems likely that a considerable amount of  $\text{Al}^{+3}$ ,  $\text{As}^{-3}$ , and  $\text{Fe}^{+2}$  has been released from these pit walls as a result of leaching and weathering processes. D-pit (Cluff) and Island 3 do not exhibit high concentrations of  $\text{Al}^{+3}$ , but are clustered into this group based on higher concentrations of Fe relative to other lakes outside of group 2.

Upper Link Lake is exposed to  $\text{Al}^{+3}$ ,  $\text{Fe}^{+2}$  and  $\text{As}^{-3}$  in a different manner. Historically, Upper Link was exposed to untreated mine runoff and currently it receives treated runoff. Mine runoff is often associated with elevated concentrations of metals, including  $\text{Fe}^{+2}$ ,  $\text{Al}^{+2}$  and  $\text{As}^{-3}$  (Nyogi et al. 2001, Pyle et al. 2001). The concentrations of  $\text{Fe}^{+2}$ ,  $\text{Al}^{+3}$  and  $\text{As}^{-3}$  in Upper Link Lake are

above background concentrations because of exposure to treated runoff from the nearby mining areas (Golder 2005).

The concentrations of indicator variables identified for group 4 were lower in Island 3 than in Island 1 and 2 (Appendix 2). Thus, Island 3 was placed in lake group 2. The concentration of Fe also increased more than an order of magnitude from 2003-2005 which likely explains the clustering of Island 3 into group 2. Reasons for the increased  $\text{Fe}^{+2}$  concentration are unknown. Major ions concentrations have decreased from 2003 to 2005 because no treated effluent has been released to Island Lake since 2002. There are other instances where lakes and pits sampled over multiple years are classified into different lake groups. The water quality within these systems appears to be transitioning (i.e. Island Lake) or fluctuating enough to be classified into different groups. Hence, I have classified each lake year as a replicate in my analyses, instead of averaging water quality variables over multiple years for a single lake. Logistics also made it impossible to sample any particular lake at the same time of the summer over multiple years. Thus, the planktonic food webs were likely at different successional stages within a given lake for a particular year. This provides additional justification for treating each year of sampling of a lake as a replicate.

There are no significant indicator variables for lake groups 1 and 3. Groups 1 and 3 are separated from group 2 by a lack of significant indicator variables, but it is not clear what separates groups 1 and 3. A separate indicator analysis of groups 1 and 3 was necessary to determine differences between these two groups. Group 3 is more similar to group 2 in the dendrogram (Fig. 2-7) therefore, an analysis of groups 1 and 2 was also necessary. The alpha level was increased to 0.10 for this analysis to identify any potential significant indicator variables between groups 1 and 2, as well as groups 1 and 3.

Aluminum and iron concentrations are much higher in group 3 than in group 1 (Table A2-2) and these may explain the separation of these groups. Several variables are associated with group 1 (Table 2-5). These results provide a basis for the separation of groups 1 and 3. When groups 1 and 2 are compared, there are a number of metals that are indicators of group 2 (Table 2-

5). Bicarbonate and pH are common indicators for group 1 when analyzed against groups 2 and 3 (Table 2-5). It is these indicators that separate group 1 from group 2. Aluminum and iron represent group 3 and group 2, while pH and bicarbonate represent group 1. However, there were several other variables that represented groups 1 and 2, which is why the cluster analysis identified 5 lake groups.

Although certain water quality variables show elevated concentrations in group 1 versus group 3 (and vice versa), these concentrations remain low when compared to the concentrations of other lake groups. The lakes and pits in groups 1 and 3 do not show any water quality impact from contamination relative to all other lakes in the data set (Table A1-1, Appendix 2). As a result, groups 1 and 3 will be merged into a single group that will be referred to as lake group 1 for the remainder of this thesis. The amalgamated group 1 lakes have negligible or no detectable water quality impact from exposure to mining activities and will be considered reference systems for the purposes of this study.

In conclusion, the resulting 4 lake groups are intended to quantify the degree and type of exposure for the study lakes, as indicated by the water quality variables, or lack thereof, associated with their respective groups. In subsequent chapters, these lake groups will be used to look for relationships between lake exposure, planktonic biodiversity and species composition, and ecosystem function.

## **CHAPTER 3. RELATIONSHIPS BETWEEN PLANKTONIC SPECIES COMPOSITION AND WATER QUALITY**

### **3.1 Introduction**

Changes in water quality are common in aquatic ecosystems that have been exposed to mining activities (Austin et al. 1985; Kalin et al. 2006) and can result in increased concentrations of metals (He et al. 2001) and salts (Moncur et al. 2006) in aquatic environments. The impacts of mining on water quality within lakes (Paktunc and Dave 2002; Pedersen 1983; Pedersen et al. 1993) and pit lakes (Early 1999) create a variety of chemical stresses that are known to affect the structure of planktonic food webs and taxonomic composition (Havens and Carlson 1998; Kalin et al. 2006; Monteiro et al. 1995). The magnitude of such effects depends on the type and degree of chemical stress (Havens and Carlson 1998; Xu et al. 2002).

Chemical stresses in aquatic communities cause shifts in trophic structure and species composition in planktonic communities (Odum 1985). However, many studies only address one or two contaminants and are often limited to single aquatic systems, or experimentally manipulated microcosms or mesocosms (Admiraal et al. 1999; Arnott et al. 1999; Carpenter 1996; Chappell and Goulder 1994; Colwell et al. 1989; Havens and Carlson 1998; Vinebrooke et al. 2003) (Maraldo and Dahllöf 2004). Very few studies deal with variation in plankton food web dynamics in response to multiple water quality variables across many aquatic systems.

Plankton samples were collected over three consecutive ice-free seasons in 2003, 2004 and 2005. Among the 26 lakes and pits sampled, 19 were exposed to various mining activities, while 7 are considered reference lakes (control lakes). The mechanisms of exposure from mining activities include surface runoff from nearby mining infrastructure, leaching from pit walls (pit lakes only), and the release of treated effluent into nearby watersheds. Relationships between water quality and species composition and abundance were sought



using multivariate ordination analysis. Such relationships become apparent if the four lake groups from chapter 2 cluster in ordination plots.

The objective of this chapter is to search for causal relationships between exposure to mining activities and planktonic species composition and abundance.

## **3.2 Methods**

### **3.2.1 Field Sampling**

Water was collected from the pelagic (deep, open-water zone) of all lakes. Temperature, depth, *Chl a*, dissolved oxygen ( $\text{mgL}^{-1}$ ), pH, specific conductivity, and TDS were measured using a YSI 6600 Sonde with a YSI 650 MDS handheld Display/Logger. Temperature profiles were used to determine the epilimnion depth (for stratified lakes only). Samples (40 L) were collected from the mid-epilimnion using an 8L Van Dorn and were placed in two 20 L polyethylene water bags. Water bags were washed and acid leached, then rinsed twice with lake water prior to sample collection. The polyethylene bags were placed in coolers and flown to the laboratory in Saskatoon. The processing of the lake water began approximately 24 hrs. after sample collection.

In addition, one litre of mid-epilimnetic water was also taken and preserved (Lugol's iodine) for plankton identification. Plankton identification was completed by either AlgaTax Consulting or Bio-Limno Research and Consulting using the Utermohls settling technique (Coulon and Alexander 1972). Data matrices of species presence-abundance (columns) and lakes (rows) in all sampling years were constructed (Table A3-1). These data matrices contained primarily phytoplankton species with a small number of small zooplankton species included (Appendix 3).

### **3.2.2 Size Structure of Planktonic Food Webs**

The size structure of the planktonic food webs were determined by measuring the quantity of phosphorus (P) in the particulate size fractions (0.2-0.8, 0.8- 2.0 and 2-40  $\mu\text{m}$ ). This was accomplished using syringe filtration with polycarbonate filters (Hudson and Taylor 2005). Larger particulate fractions (40-

200 and >200  $\mu\text{m}$ ) were determined with nylon screens with a serial filtration approach (Hudson and Taylor 2005). The amount of water used for serial filtration ranged from 1 L to 12 L because some lakes required more water to be filtered in order to obtain a noticeable concentration of organisms on the filter, thus providing a more precise estimate of P in the larger particulate fractions.

The total phosphorus (TP) in the size fractions was used to determine the distribution of biomass through the food web (e.g. missing size fractions or dominant fractions). Total phosphorus for all lakes was calculated as the sum of dissolved and particulate P (Parsons et al. 1984). Phosphorus was analysed colorimetrically using the molybdenum blue technique with persulfate oxidation (Menzel and Corwin 1965).

### **3.2.3 Mantel Tests**

Mantel Tests were used to test for similarity in species composition and abundance among the data matrices for each year. The null hypothesis of no similarity between two symmetrical data matrices was tested. The data matrices are symmetrical in that they contain the same samples (lakes), however, the number of species present (plankton) varies. Data matrices were constructed using lakes and pits common among all three sample years. Three pair-wise analyses were completed for all sampling years (2003-2005) using a randomized Monte Carlo version of the Mantel Test. The Monte Carlo version switches the rows and columns of one of the two data matrices and produces a test statistic showing how many times a correlation equal to or more extreme than the observed value could be determined. This information is used together with the number of randomizations to calculate a p-value that indicates the degree of association between the two data matrices (McCune and Grace 2002).

### **3.2.4 Canonical Correspondence Analysis (CCA)**

The word “canonical,” in statistical terms, refers to methods of determining the underlying structure in two or more data matrices simultaneously. CCA constrains an ordination of one matrix (i.e. species composition and abundance) by a multiple linear regression on a matrix containing the environmental variables

for the same samples (McCune and Grace 2002). Thus, CCA has the potential to show how species composition and abundance data are structured by the measured environmental variables. However, for CCA to be effective the environmental gradients controlling species abundance must be measured. CCA attempts to answer the question, how much variation in species presence and abundance is directly explained by the environmental variables?

CCA does have some weaknesses. For example, due to the multiple regression technique, CCA results become less reliable as the sample size (number of lakes) decreases in comparison to the number of environmental variables measured. If sample size is too low, strong relationships can be found using random number predictors which is clearly undesirable. To avoid this problem, a prior Principal Components Analysis (PCA) was necessary to reduce the number of environmental variables to a smaller number of synthetic variables appropriate for CCA analysis.

Principal Components Analysis (PCA) is the oldest and most basic form of eigenvector analysis, dating as far back as 1901 (McCune and Grace 2002). The purpose of PCA is to reduce a data set containing many variables to a data set explained by a small number of synthetic variables that represent the strongest correlations found in the data. These synthetic variables are the principal components, the ordination axes typically used to illustrate results. PCA is a linear model that only works well with data that show a linear response to the variables that are being measured.

The 24 measured environmental variables were condensed by performing PCA on the environmental matrices for the separate sample years. Lake scores for the first three statistically significant axes from the PCA analyses were used to construct new, synthetic environmental data matrices for each sample year. Each PCA axis represents a complex (synthetic) variable that explains as much variation as possible in the water quality data matrix. For example, PCA analysis on the 2003 environmental data revealed that TDS,  $\text{Ca}^{+2}$ , and  $\text{Mg}^{+2}$  were strongly correlated with axis 1 (Table 3-1). Thus, if axis 1 from the PCA shows a strong correlation with axis 1 in the CCA ordinations, then one can conclude that

TDS,  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  explain the distribution of lakes and species along axis 1 of the CCA

**Table 3-1.** Water quality variables correlated with the significant axes from PCA analysis of each sample year. These axes were used as synthetic environmental variables in CCA analysis.

Axis	2003	2004	2005
<b>AXIS1PCA</b>	TDS, $\text{B}^{+3}$ , $\text{Ca}^{+2}$ , $\text{Cl}^-$ , $\text{Mg}^{+2}$ , $\text{SO}_4^{-2}$ , Hardness	TDS, $\text{B}^{+3}$ , $\text{Ca}^{+2}$ , $\text{Cl}^-$ , $\text{Mg}^{+2}$ , $\text{Na}^+$ , $\text{SO}_4^{-2}$ , Hardness	$\text{B}^{+3}$ , $\text{Ca}^{+2}$ , $\text{Cl}^-$ , $\text{K}^+$ , $\text{Mg}^{+2}$ , $\text{Na}^+$ , $\text{SO}_4^{-2}$ , Hardness
<b>AXIS2PCA</b>	$\text{Mn}^{+2}$ , $\text{Ni}^{+2}$ , $\text{Se}^{-2}$ , $\text{Zn}^{+2}$	$\text{As}^{-3}$ , $\text{Cu}^{+2}$ , $\text{Ra-226}^{+2}$ , $\text{Zn}^{+2}$	$\text{Al}^{+3}$ , $\text{Cu}^{+2}$ , $\text{Fe}^{+2}$ , $\text{HCO}_3^-$
<b>AXIS3PCA</b>	$\text{HCO}_3^-$ , $\text{Ra-226}^{+2}$ ,	$\text{Al}^{+3}$ , $\text{Fe}^{+2}$	$\text{Ba}^{+2}$ , $\text{Cu}^{+2}$ , $\text{U}^{+3}$ , $\text{Ra-226}^{+2}$

ordination. CCA was performed on the species data for each sampling year separately because different lakes were sampled in different years. Therefore, the community matrices from separate years could not be combined.

Prior to analysis, species abundance data were transformed ( $\text{LOG}(x+1)$ ) for all years to improve skewness and kurtosis of the data. Outlier analysis was then performed on lakes and species (2 standard deviation cut-off) and both such outliers were deleted from the data matrix before CCA analysis. These Species present in only two or fewer lakes were also deleted. Due to the mathematical treatment of rare and outlying lakes and species in CCA and NMDS, this type of data modification is necessary to provide the most meaningful ordination results (McCune and Grace 2002).

The lakes on the ordinations are accompanied by symbols representing their respective groups. It is important to remember that group 1 on the ordinations represents a combination of the lakes in groups 1 and 3 from Chapter 2. These two groups had no significant indicator variables associated with them and represent a set of lakes that are considered non-impacted in terms of water quality. The purpose of overlaying the lake groups, derived from cluster analysis

of environmental variables, onto the ordinations is to assess whether or not they cluster based on ordinations of species presence-abundance data. Such results would indicate direct relationships between water quality and phytoplankton species composition and abundance.

CCA is considered a “direct gradient analysis” due to the simultaneous analysis of the species matrix and the water quality matrix. Direct gradient analysis ordines species and samples (lakes) in environmental space. NMDS analyzes only the species data matrix and ordinations are in species space. Environmental correlations, with the reduced number of dimensions, are determined separately in NMDS, making it an indirect gradient analysis. Despite the theoretical differences between these two ordinations, results should show a general correspondence if the controlling environmental variables have been included.

### **3.2.5 Non-metric Multidimensional Scaling (NMDS)**

Non-metric Multidimensional Scaling is the second multivariate method chosen for analysis of species composition and abundance data. NMDS was chosen because, as previously explained, it is a fundamentally different ordination technique from CCA and is intended to act as an independent assessment of the CCA results.

NMDS is a non-metric technique, meaning that linear assumptions (present in PCA and CCA) are absent because rank distances are used, rather than correlation or regression coefficients (McCune and Grace 2002). This use of rank distances also alleviates the zero truncation problem present in most other community ordination techniques. A non-metric function is preferred when considering species data because species generally show a unimodal, or Gaussian, response to environmental variables. Because of these differences from other ordination techniques, NMDS is frequently the ordination technique of preference chosen for ecological community data sets (McCune and Grace 2002; Morabito et al. 2003; Brehm and Fiedler 2004).

Another feature of NMDS is its use of a stress function, which is the difference between distances within the original data and distances in the ordination with its reduced number of dimensions. Stress is a measure of departure from monotonicity among the original multi-dimensional space and the reduced ordination dimensional space. Low stress indicates that the ordinations produced by a particular analysis are a good representation of the original data. Any type of distance measure can be used, however Sorensen distance is recommended for ecological community data (McCune and Grace 2002).

### **3.2.6 Indicator Species Analysis**

Indicator Species Analysis (equivalent to Indicator Analysis of Chapter 2) was used to determine if any species showed significant associations with any of the four lake groups. This analysis was used to search for indicator species for each lake group that were common across all sample years. This would determine if certain species were representative of certain types of lake exposure. Separate analyses were also conducted for each sampling year because not all lakes and pits were sampled each year. Also, lakes were not necessarily sampled at the same time in each season with the result that species composition and abundance could be expected to be the same in each sample year. Thus, a separate analysis was conducted for each sample year.

### **3.2.7 Multi-Response Permutation Procedures (MRPP)**

MRPP (refer to Chapter 2) were used to determine if species composition and abundance were significantly different between the four lake groups. MRPP analysis was performed on all groups from each sampling year. Pair-wise analysis between the lake groups was not possible because there were instances in all sampling years where only one or two lakes were present in certain groups, which invalidates the analysis. Regardless, MRPP of all lake groups shows whether or not groups within a particular year are significantly different in their species composition and abundance.

### **3.3 Results**

#### **3.3.1 Comparison of Annual Species Matrices**

Mantel test results of the species matrices from each sample year are as follows: 2003 vs. 2004,  $r = 0.325$ ,  $p = 0.02$ ; 2003 vs. 2005,  $r = 0.211$ ,  $p = 0.12$ ; 2004 vs. 2005,  $r = 0.256$ ,  $p = 0.06$ . A significance level of 0.10 was used for this analysis due to the heterogeneity of the species matrices. Note that the 2003 and 2005 species matrices were not significantly correlated at the 0.10 alpha level, although the relationship was very close to being significant. This suggests that all species matrices are similar in terms of species presence and abundance. Within the limits of the reduced data set analysed (see methods), the lake and pit planktonic food webs do not vary in a random fashion from year to year.

#### **3.3.2 Relationships between Water Quality and Phytoplankton Composition and Abundance**

##### 2003

All of the lake groups cluster relatively well, except for group 4 (Fig. 3-1). Island Lake, in particular, is isolated from the other two group members, as well as all other lakes. The total variance explained by CCA over the two ordinated axes was 22.3% (Table 3-2). Only axis 1 is representative of the original data due to its significant relationship between the species and environment matrices (Monte Carlo test, Table 3-2) and high correlation between raw-data distance and ordination distance (0.236) relative to axes 2 (0.002) and 3 (-0.047).

Lakes that are positioned on the right side of axis 1 of the CCA ordination have higher concentrations of the variables associated with this axis, compared to lakes that are positioned on the left side of axis 1 and near the origin.

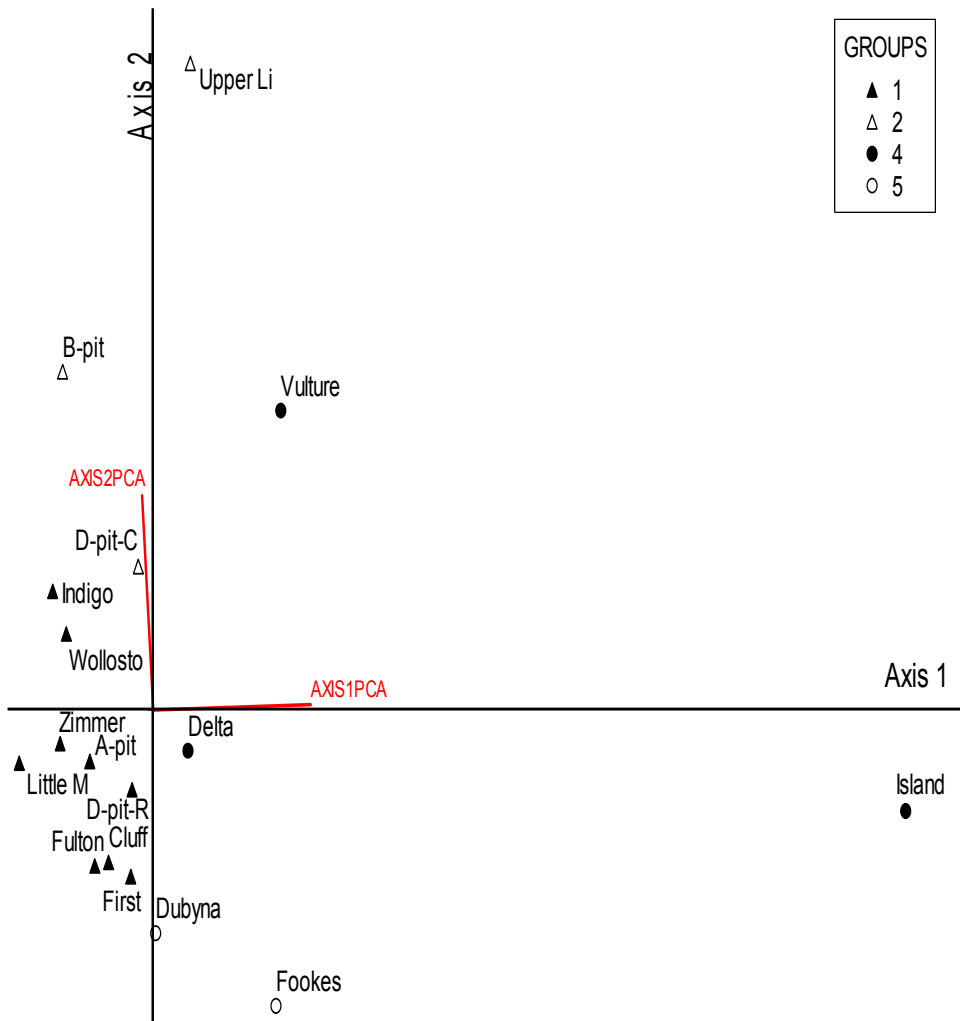
AXIS1PCA, the synthetic variable derived from PCA, shows a strong positive correlation with axis 1 in Fig. 3-1 (biplot correlation of 0.907, Table 3-2). The environmental variables that are associated with AXIS1PCA are total dissolved solids (TDS), boron, calcium, chlorine, magnesium, sulfate, and hardness (Table 3-1) and these variables explain the positioning of the lakes along axis 1. Island Lake clearly dominates the axis, being positioned at the extreme right-hand side

of the ordination (Fig. 3-1) and it contains the greatest concentrations of TDS and sulphate. This can be seen in Fig. 3-2, which is the same plot as Fig. 3-1 except that symbol sizes associated with the lake groups vary with the concentrations of the variables associated AXIS 1 from the CCA environment matrix (Table A1-1). Vulture and Delta Lake, also belonging to group 4, have concentrations of AXIS1PCA environmental variables that are lower, but are nonetheless larger than for all other lakes.

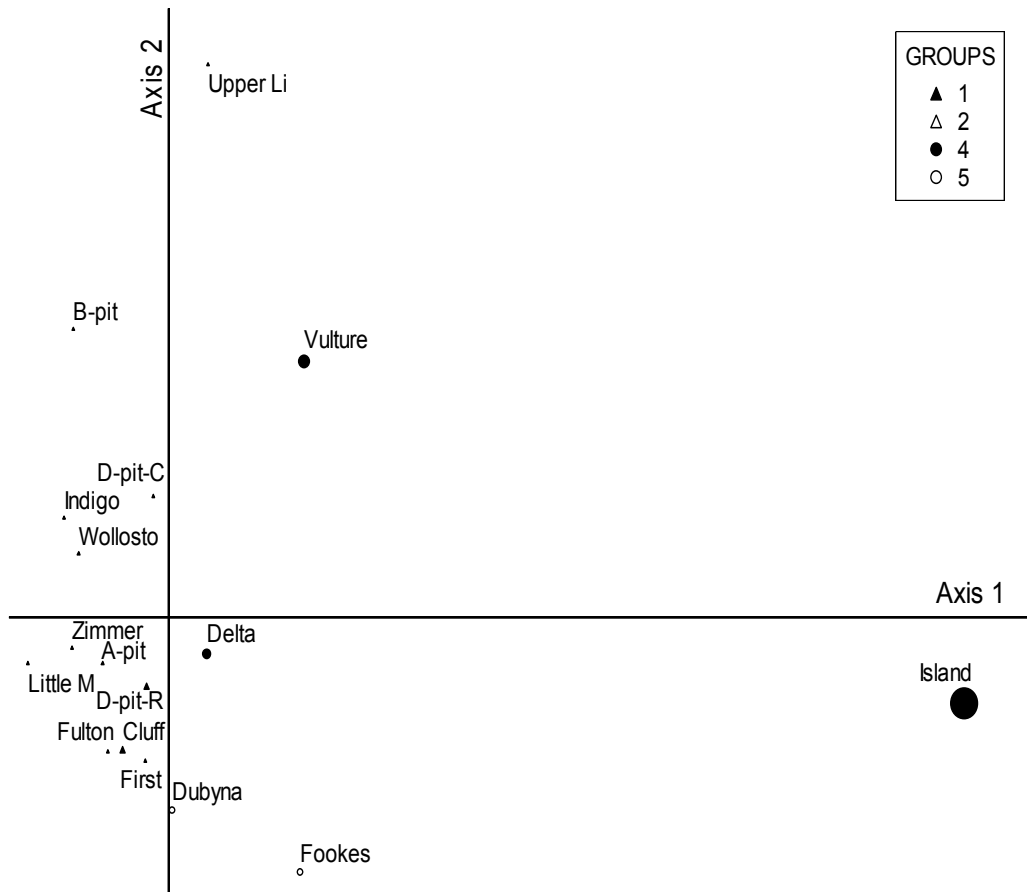
The species ANKIST-F (*Ankistodesmus falcatus*), CHROOC-M (*Chroococcus minutes*) and TETRAED (*Tetraedron minimum*) (Table A3-1) are positioned far along the right side of axis 1 (Fig. 3-3) and are therefore abundant in Island Lake, which occupies a similar position in the ordination of lakes (Fig. 3-1). Sue-C pit was identified as an outlier, in terms of species composition and abundance, for the 2003 and was removed from the 2003 species matrix prior to ordination analysis.

NMDS was performed on the 2003 species abundance data to provide an independent assessment of the CCA results. The NMDS scree plot for the 2003 species data (Fig. 3-4) shows an elbow at 2 dimensions and indicates that a 2-dimensional solution is appropriate. Minimum, maximum, and mean stress values, based on random number runs, are plotted in Fig. 3-4 for comparison with stress values calculated from real data. All recommended dimensions (axes) are significantly below randomized data runs ( $\alpha \leq 0.05$  – Monte Carlo Test for significance).

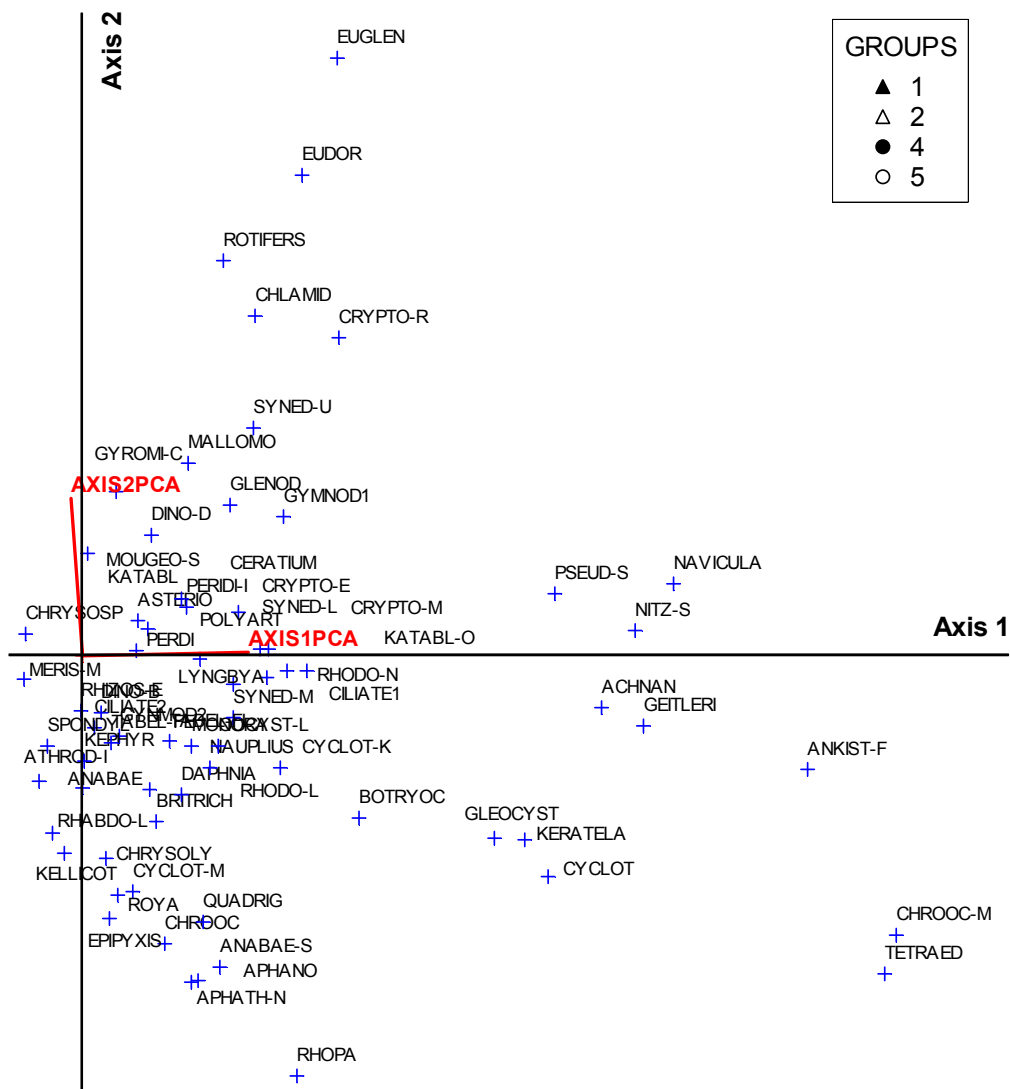




**Figure 3-1.** 2003 CCA ordination biplot of lakes plotted in environmental space based on species composition and abundance data. Lake groups cluster relatively well and AXIS1PCA explains the positioning of lakes along axis 1. AXIS1PCA is a synthetic variable that represents total dissolved solids (TDS), boron, calcium, chlorine, magnesium, sulphate and hardness (Table 3-1). Axis 1 is significant, while axis 2 is non-significant, as determined by CCA analysis (Table 3-2). AXIS2PCA appears to show a positive correlation with axis 2. However, this is only because the axes in this ordination are inverted. AXIS2PCA in fact has a negative correlation with axis 2 as shown in Table 3-2.



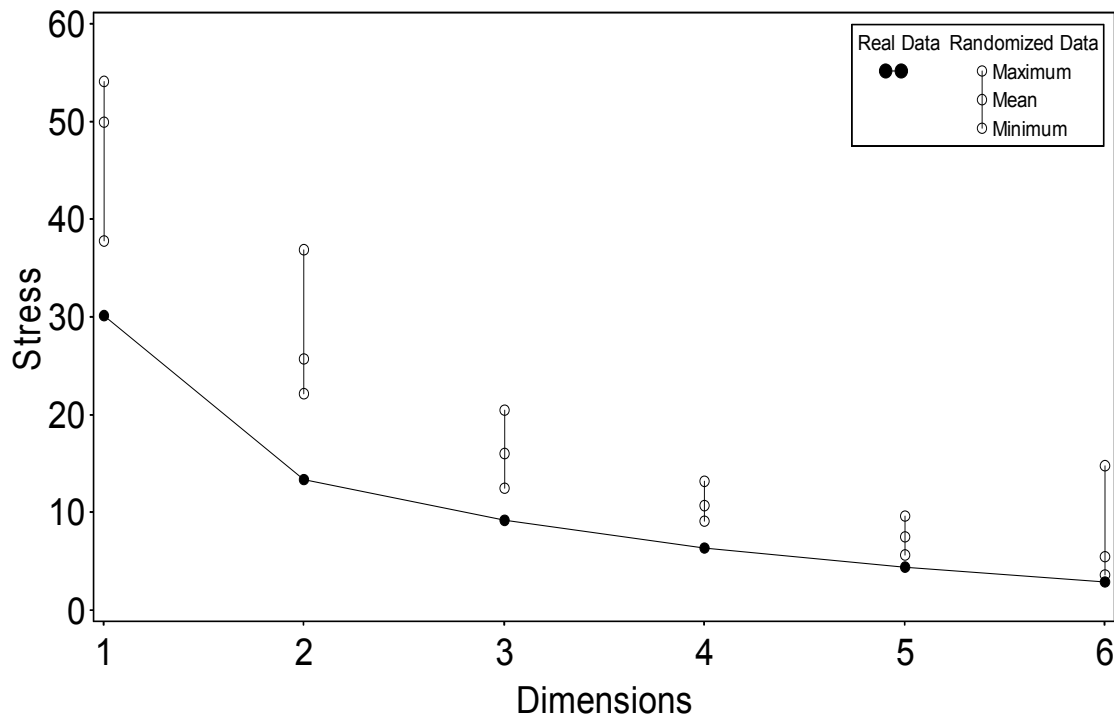
**Figure 3-2.** CCA ordination of 2003 species abundance data. This figure is the same as figure 3-1, but shows the relative concentrations of the variables represented by the first synthetic variable (derived from PCA of the 24 environmental variables). Island Lake is positioned at the far right of the ordination due to its high concentrations of the variables represented by AXIS1PCA (total dissolved solids (TDS), boron, calcium, chlorine, magnesium, sulphate and hardness, Table 3-1).



**Figure 3-3.** 2003 CCA ordination of species plotted in environmental space. Species positioned on the right side of axis 1 are tolerant to total dissolved solids (TDS), boron, calcium, chlorine, magnesium, sulphate and hardness. Species positioned similarly to lakes in Fig. 3-1 are abundant in those lakes and are tolerant to the water quality conditions within their respective lakes. AXIS2PCA appears to show a positive correlation with axis 2. However, this is only because the axes in this ordination are inverted. AXIS2PCA in fact has a negative correlation with axis 2 as shown in Table 3-2.

**Table 3-2.** Summary of results from CCA of the 2003 species abundance data. Variance explained (%) is the highest for axis 1. Significant p-values associated with the Monte Carlo results indicate a strong relationship between the species data matrix and the environmental data matrix ( $\alpha = 0.05$ ). Correlation between raw-data distance and ordination distance indicate a good representation of the original data for axis 1. Biplot correlations show the strength of relationships between the environmental measurements and the ordination axes (representing the species data).

	Axis	Increment	Cumulative	
Variance explained (%)	<b>1</b>	<b>12.9</b>	<b>12.9</b>	
	2	9.4	22.3	
	3	6.1	28.4	
Correlations between raw-data distance and ordination distance	<b>1</b>	<b>0.236</b>	<b>0.236</b>	
	2	0.002	0.238	
	3	- 0.047	0.191	
Monte Carlo Results (p-values)	<b>1</b>	<b>0.012</b>		
	2	0.472		
	3	0.564		
Biplot Correlations from CCA		AXIS1PCA	AXIS2PCA	AXIS3PCA
	1	<b>0.907</b>	-0.263	- 0.266
	2	0.288	-0.822	- 0.255
	3	- 0.232	-0.237	0.823



**Figure 3-4.** NMDS Scree plot based on 2003 species data. The sharp decrease in stress over the first two dimensions suggests that a 2-dimensional (axis) solution is appropriate for this data set. Plots for real data runs are well below the means of the randomized runs for all recommended solutions and all real-run plots are significantly different from random-run plots ( $p \leq 0.05$ ).

Planktonic species composition and abundance are similar among lakes within each individual group that was classified according to the water quality variables. This is shown in the NMDS ordination (Fig. 3-5) by the clustering of lake groups similar to that in CCA, which provides additional confidence in the results of both analyses. Although clustering is not as tight, Island Lake is again positioned on the far right-hand side of axis 1 (Fig. 3-5). Many of the same variables correlated with AXIS1PCA in CCA are also strongly correlated with axis 1 of the NMDS ordination. Correlations between ordination distances and distances in the original data for axes 1 and 2 were 0.502 and 0.339, respectively (Table 3-3). This indicates that axis 1 represents more information (and accounts for the most variation) in the original data than axis 2.

Cumulative correlation between raw data distance and ordination distance, among axes considered for interpretation, is much lower for CCA (0.236, Table 3-

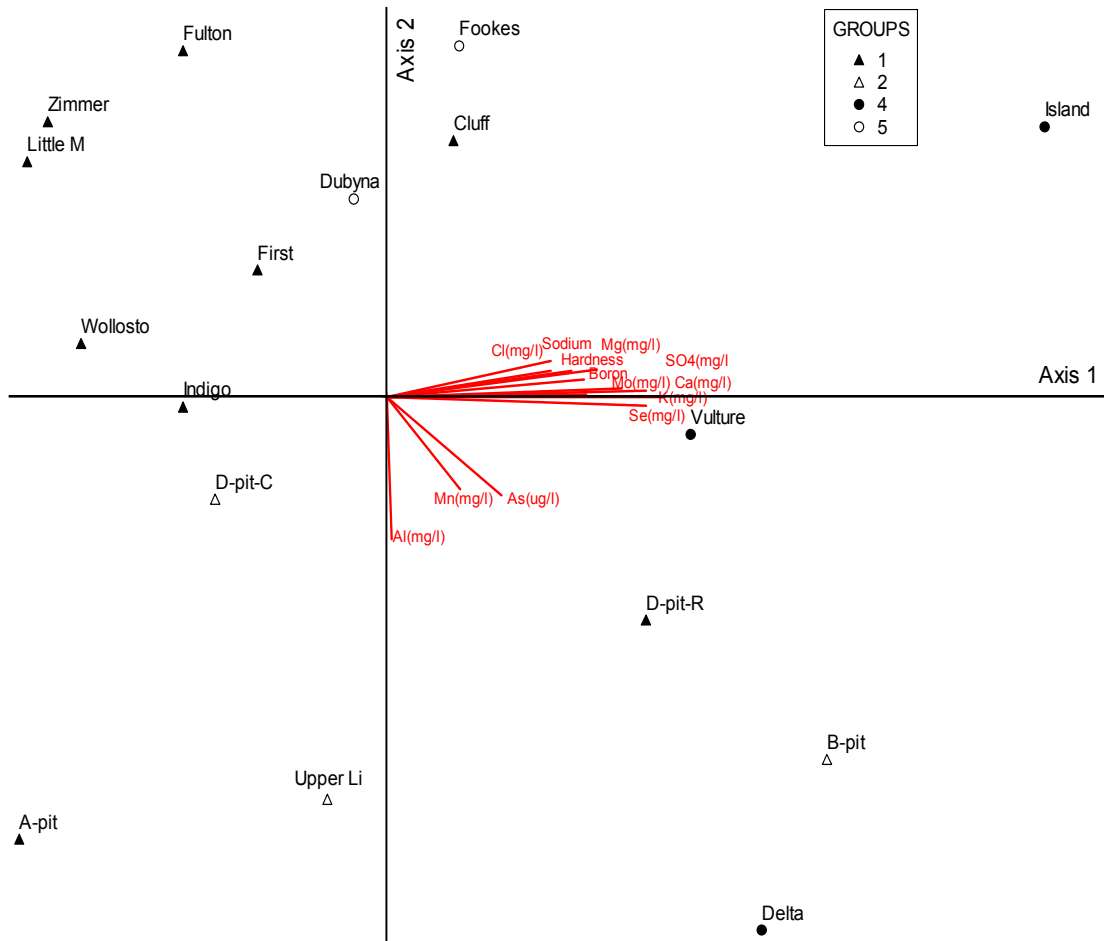
2) than for NMDS (0.841, Table 3-3). NMDS captures additional variation that was not accounted for by CCA, implying that there are other factors affecting the phytoplankton community aside from the water quality variables measured in this study.

The positioning of species in Fig. 3-6 is also similar to those positioned in Fig. 3-2 (CCA results). Overall, results from NMDS compare well with those from CCA.

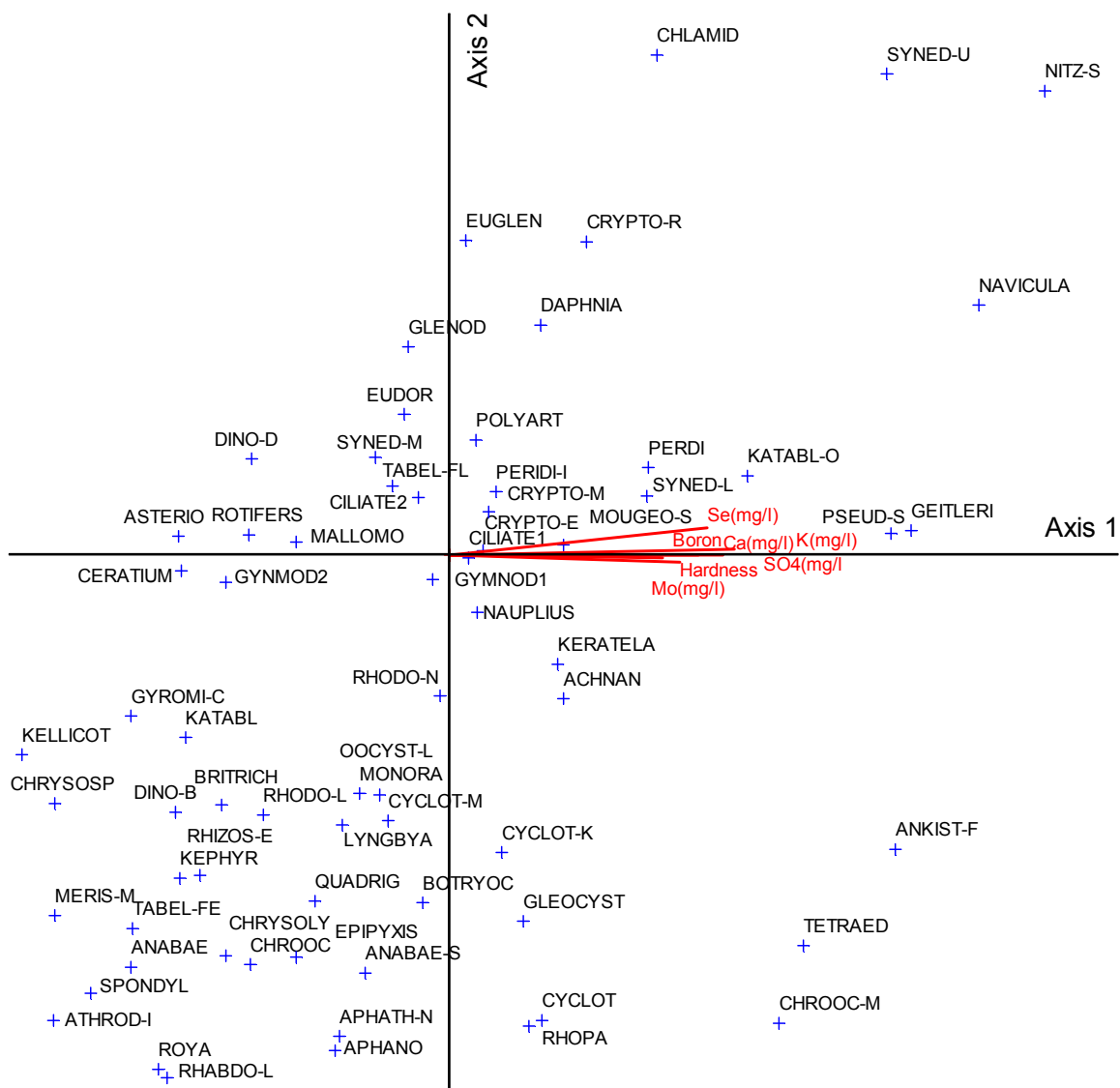
MRPP results for all four lake groups are significantly different from one another (MRPP,  $p$ -value = 0.008) in terms of species composition and abundance. There is strong separation between groups as indicated by the negative test statistic (MRPP,  $T = -2.66$ ). Chance corrected within-group agreement is greater than 0.1 (MRPP,  $A = 0.2$ ) indicating that within-group lakes are more similar to one another than expected by chance.

#### 2004

Species composition and abundance appears to be quite similar among the lakes and pits of group 1, the group forming a tight cluster near the origin of the CCA ordination (Fig. 3-7). Dubyna Lake is the only group 5 representative for 2004 and is positioned similarly to group 1 lakes. This implies that species composition and abundance in Dubyna Lake is similar to that in group 1 lakes. Lake group 2 does not cluster as well, indicating that species composition and abundance are more variable in this group. Group 4 lakes are positioned similarly along axis 1, but are separated on axis 2.



**Figure 3-5.** 2003 NMDS ordination of species abundance data. Clustering is similar to more diffuse CCA results presented in Fig. 3-1. Many of the variables contained in AXIS1PCA, from the CCA environmental matrix, are correlated with axis 1 of the NMDS ordination.



**Figure 3-6.** 2003 NMDS ordination of species abundance. The positioning of species' along axis 1 are very similar to those in Fig. 3-3 indicating that NMDS results are comparable to CCA results.

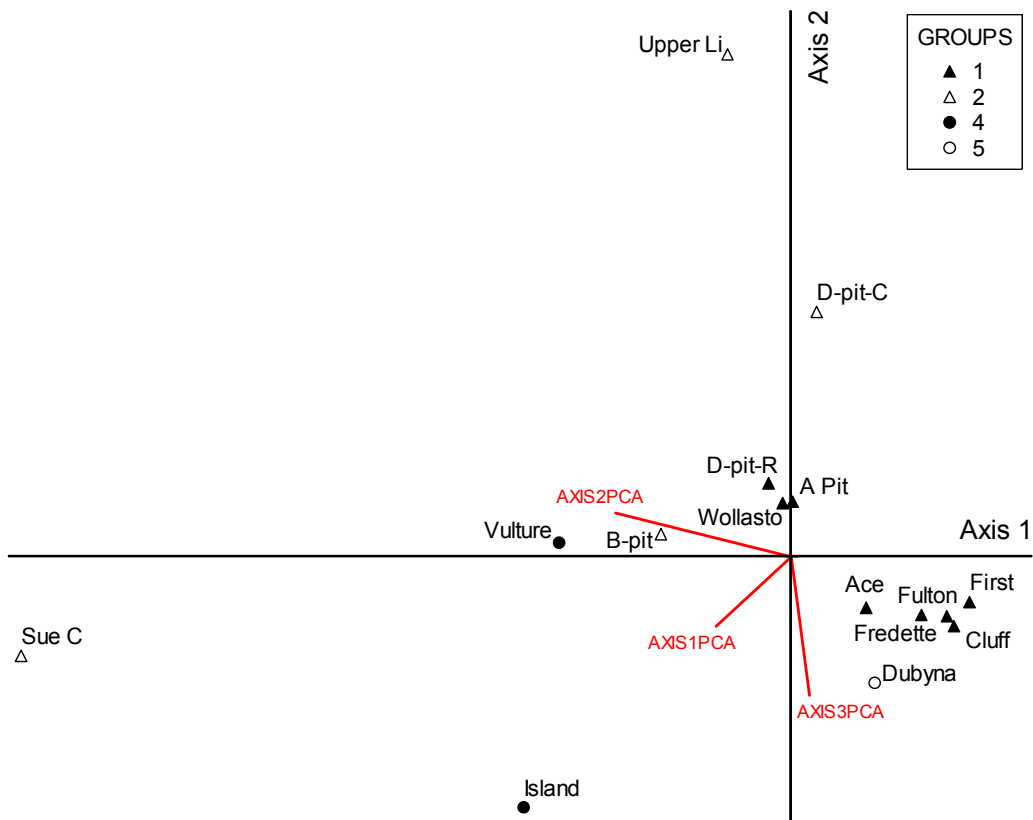


**Table 3-3.** Summary Statistics from NMDS of 2003 species composition data. Axis 1 is most representative of the raw data as indicated by its higher correlation between raw data distance and ordination distance, although both axes are significant. The environmental variables that have the highest correlations with axis 1 are similar to those represented by AXIS1PCA from CCA indicating that NMDS and CCA results are comparable.

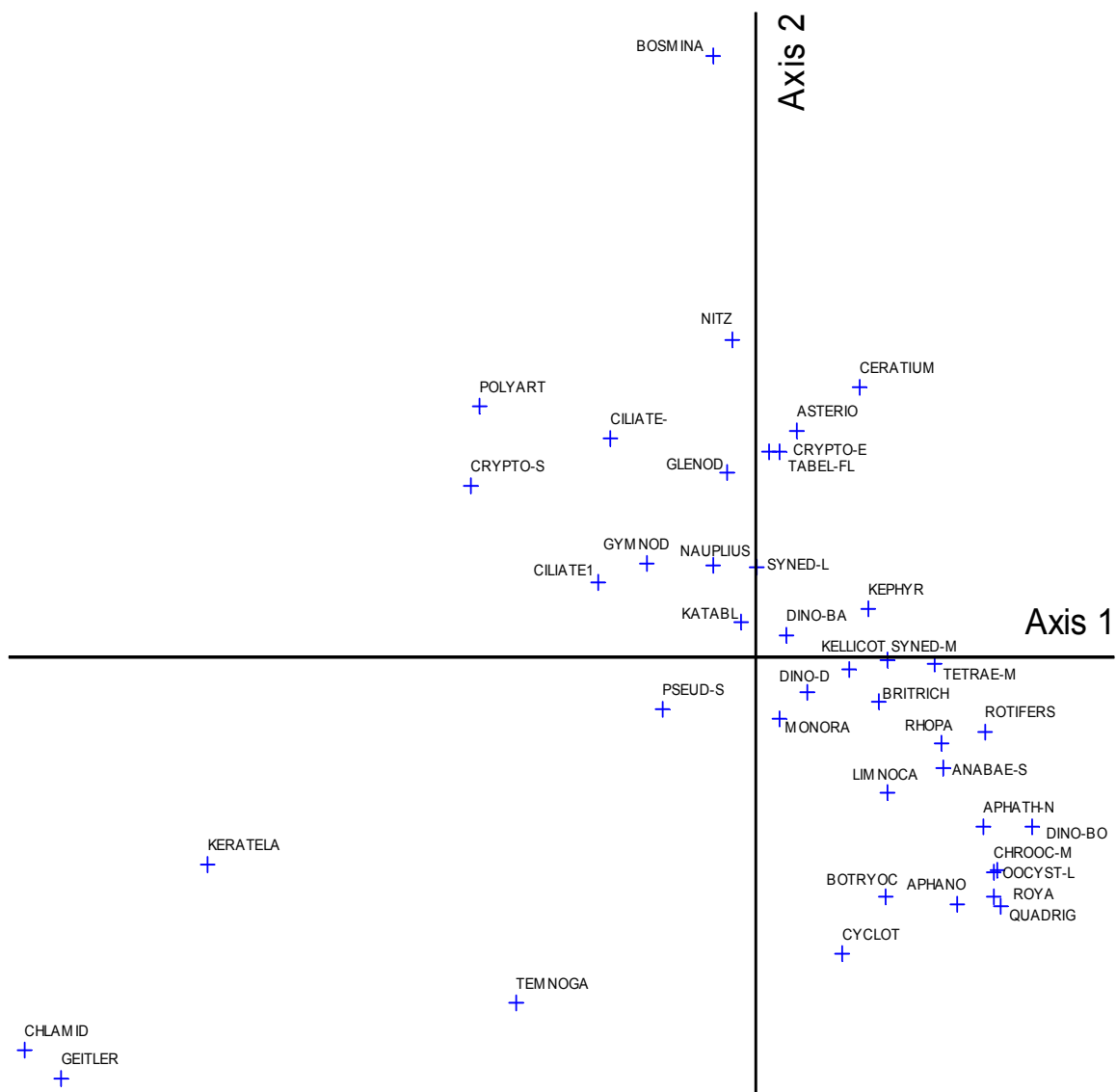
	Axis	Increment	Cumulative
Correlations between raw-data distance and ordination distance	1	<b>0.502</b>	0.502
	2	<b>0.339</b>	0.841
Highest correlations with environmental variables	1	K <sup>+</sup> – 0.540 Ca <sup>+2</sup> – 0.506 Se <sup>-2</sup> – 0.506 Hardness – 0.410	
	2	Al <sup>+3</sup> - 0.256	

The positioning of lakes along axis 1 is influenced by the water quality variables represented by AXIS2PCA, which shows the strongest correlation with axis 1 (Table 3-4). These variables are arsenic (As<sup>-3</sup>), copper (Cu<sup>+2</sup>), radium-226 (Ra-226<sup>+2</sup>), and zinc (Zn<sup>+2</sup>) (Table 3-1). Only axis 1 was considered for interpretation due to its high variance explained and the high correlation between raw-data distance and ordination distance (Table 3-4). Monte Carlo results indicate that only axis 1 showed a significant relationship between the species and environment matrices. AXIS1PCA is aligned with Island lake, as it was in 2003, and is again associated with higher concentrations of TDS, B<sup>+3</sup>, Ca<sup>+2</sup>, Cl<sup>-</sup>, Mg<sup>+2</sup>, Na<sup>+2</sup>, SO<sub>4</sub><sup>-2</sup>, and Hardness relative to other lakes.

An ordination of species plotted in environmental space (Fig. 3-8) shows that *Chlamydomonas* spp. and *Geitlerinema* spp. are abundant in Sue-C pit as indicated by their similar positioning in the ordination as Sue-C pit. *Bosmina* spp. is abundant in Upper Link Lake. These species are clearly adapted to the water quality within their respective lake or pit. McClean Lake was considered an outlier in 2004, based on species composition and abundance, and was removed from the data set prior to ordination analysis.



**Figure 3-7.** 2004 CCA ordination biplot of lakes plotted in environmental space based on species composition and abundance data. Lake groups 1 and 5 cluster tightly, indicating that species composition and abundance is similar among these lakes. Lake groups 2 and 4 are positioned on the left side of axis 1 and are associated with **AXIS2PCA**. This axis is a synthetic variable that represents arsenic ( $\text{As}^{-3}$ ), copper ( $\text{Cu}^{+2}$ ), radium-226 ( $\text{Ra-226}^{+2}$ ), and zinc ( $\text{Zn}^{+2}$ ).



**Figure 3-8.** 2004 CCA ordination of species plotted in environmental space. Species positioned on the left side of axis 1 are tolerant to arsenic ( $\text{As}^{-3}$ ), copper ( $\text{Cu}^{+2}$ ), radium-226 ( $\text{Ra-226}^{+2}$ ), and zinc ( $\text{Zn}^{+2}$ ). Species positioned similarly to the lakes in Fig. 3-7 are abundant in those lakes and are tolerant to the water quality conditions within their respective lakes.

NMDS output for 2004 species data suggests that a three dimensional solution is appropriate for this data set. (Fig. 3-9, elbow at 3 dimensions). The lake groups again do not cluster as tightly in NMDS (Fig. 3-10) compared to CCA (Fig. 3-7). This is particularly the case with lake group 1, which shows much more separation in the NMDS ordination (Figure 3-10) than in the CCA ordination (Fig. 3-7).

**Table 3-4.** Summary results from CCA of the 2004 species abundance data. Variance explained (%) is the highest for axis 1. Significant p-values associated with the Monte Carlo results indicate a strong relationship between the species data matrix and the environmental data matrix ( $\alpha = 0.05$ ). High correlations between raw-data distance and ordination distance indicate a good representation of the original data for axis 1 only. Biplot correlations show the strength of relationships between the environmental measurements and the ordination axes (representing the species data). Values that are ideal for interpretation are bolded and show that only axis 1 should be considered for interpretation.

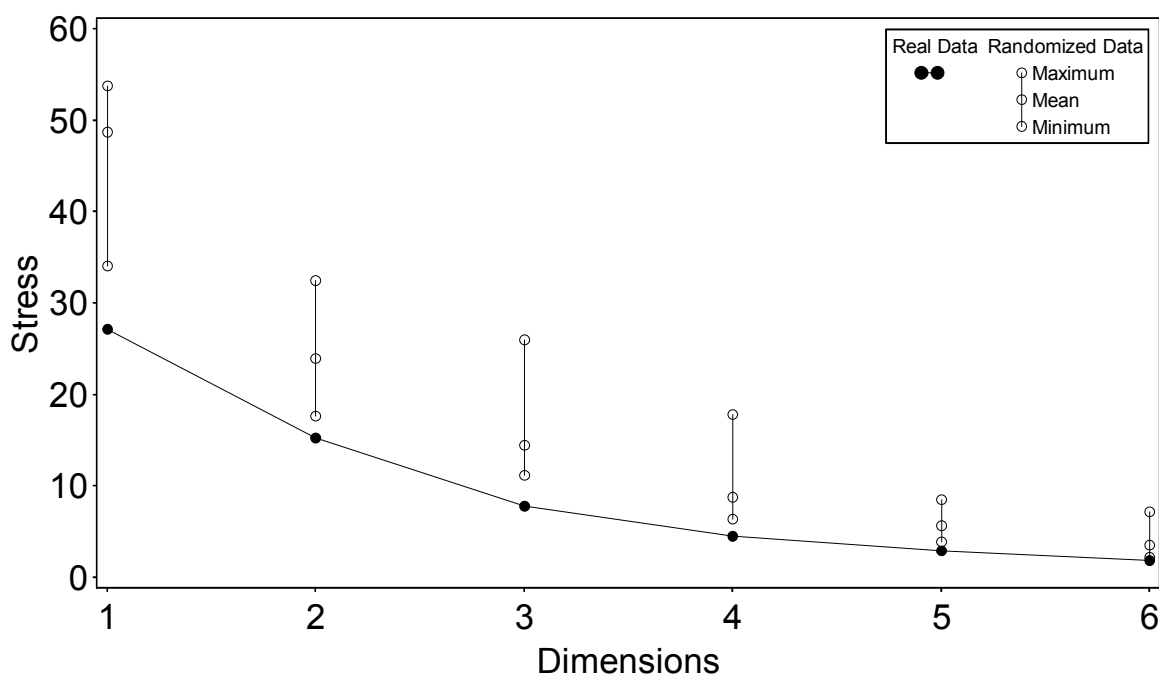
	Axis	Increment	Cumulative	
Variance explained (%)	<b>1</b>	<b>20.4</b>	<b>20.4</b>	
	2	9.7	30.1	
	3	5.6	35.7	
Correlations between raw-data distance and ordination distance	<b>1</b>	<b>0.635</b>	<b>0.635</b>	
	2	- 0.065	0.570	
	3	- 0.012	0.558	
Monte Carlo results	<b>1</b>	<b>0.002*</b>		
	2	0.436*		
	3	0.060*		
Biplot correlations		AXIS1PCA	AXIS2PCA	AXIS3PCA
	<b>1</b>	- 0.398	<b>-0.928</b>	0.095
	2	- 0.440	0.284	-0.885
	3	0.805	-0.240	-0.456

\*Values are p-values resulting from a Monte Carlo test

This suggests again that there are other environmental variables, not accounted for by CCA, that are influencing the species composition and abundance of these lakes and pits. However, many of the same lakes that were positioned at the extremes of the axes in CCA (Island Lake, Sue-C pit, D-pit Cluff) also show up at the extremes of the axes in NMDS (Fig. 3-10). The environmental variables that explain the positioning of the lakes in NMDS are similar to those in CCA.

Species positions in environmental space (Fig. 3-11) are similar to those in CCA (Fig. 3-8). For example, *Chlamydomonas* spp. and *Geitlerinema* spp. are again abundant in Sue-C pit. Axes 1 and 3 best represent the raw species composition data for 2004 as shown by their high distance preserving properties (Table 3-5). Based on these results, axes 1 and 3 were considered for interpretation. Among axes considered for interpretation, correlations between raw data distance and ordination distance were higher for NMDS (0.808, Table 3-5) than for CCA (0.635, Table 3-2).

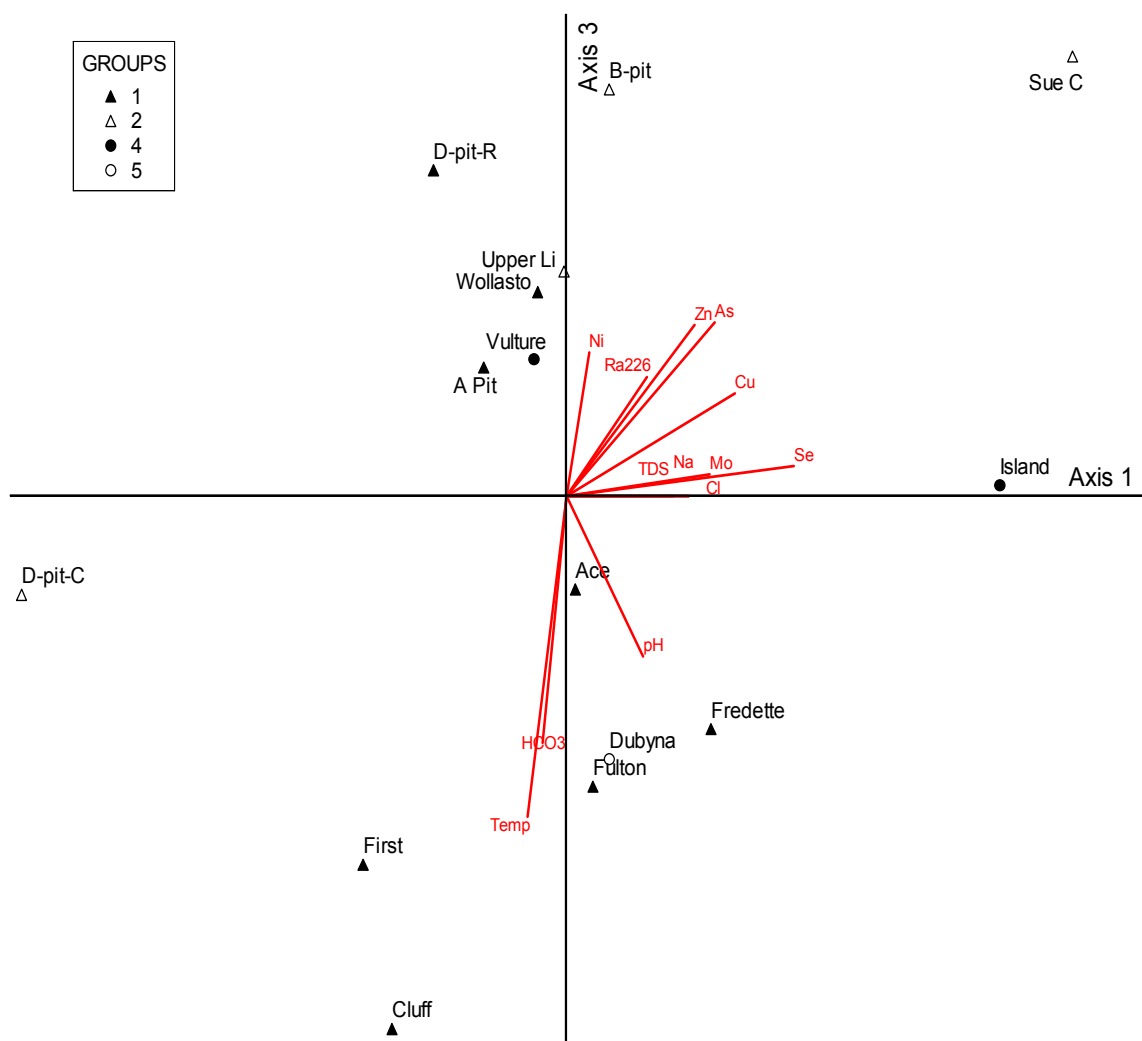
No significant difference in species composition and abundance was found between lake groups (MRPP,  $p = 0.08$ ,  $T = -1.45$ ,  $A = 0.119$ ) in 2004, although the  $p$ -value would be considered significant at the 10% level. This suggests that there are likely other environmental variables, not measured in this study, which are influencing species composition and abundance in the lakes and pits.



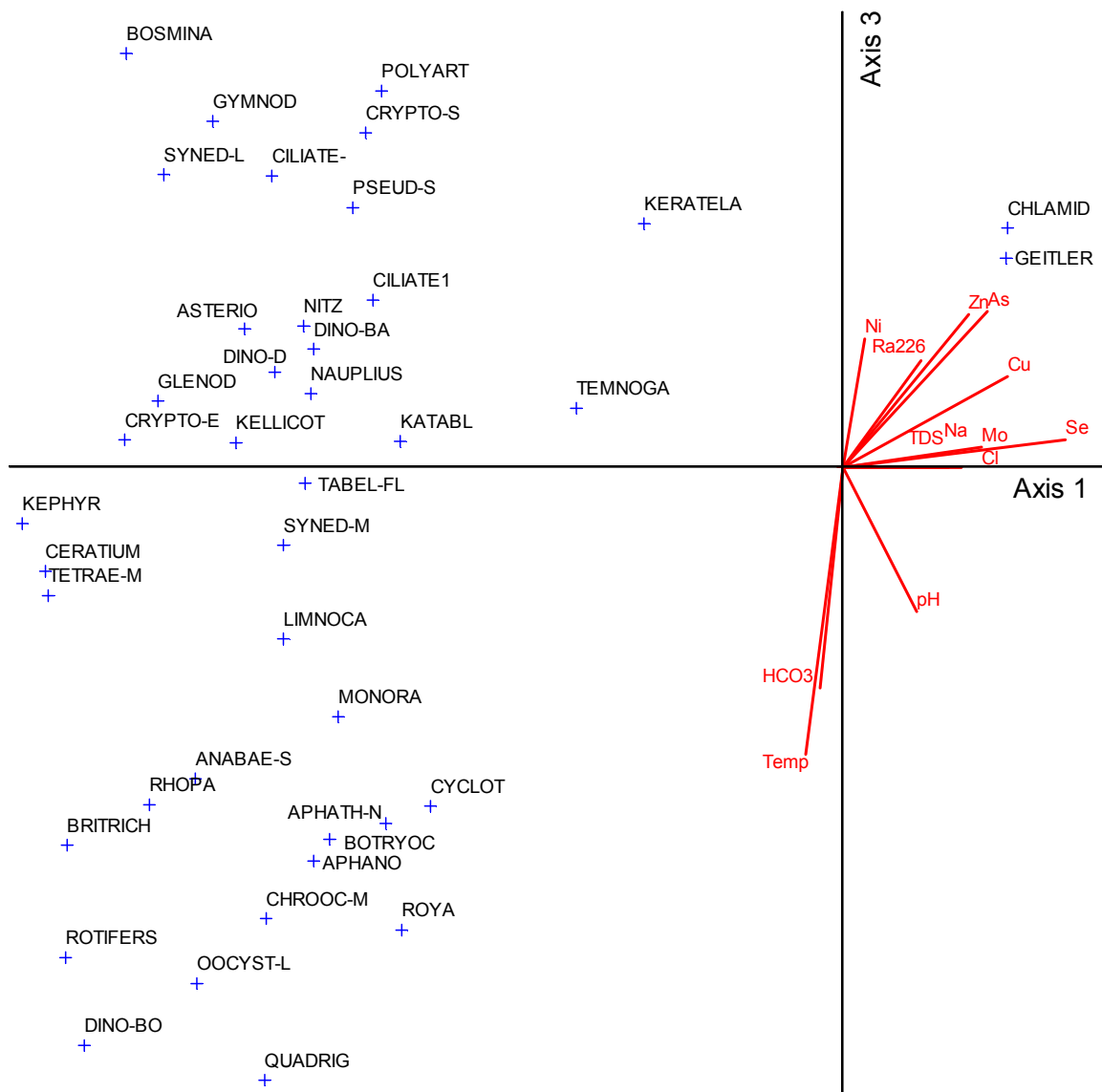
**Figure 3-9.** NMDS Scree plot based on species data from 2004. The sharpest decrease in stress over the first three dimensions suggests that a 3-dimensional (axis) solution is appropriate for this data set. Plots for real data runs are well below the means of the randomized runs for all recommended solutions and all real-run plots are significantly different from random-run plots ( $p \leq 0.05$ ).

**Table 3-5.** Summary Statistics from NMDS of 2004 species composition data. Axes 1 and 3 are more representative of the raw data as indicated by their higher correlations between raw data distance and ordination distance. The environmental variables that have the highest correlations with axes 1 and 3 are most similar to those represented by AXIS2PCA from CCA (Table 3-1), meaning that NMDS and CCA results are comparable.

	Axis	Increment	Cumulative
Correlations between raw data distance and ordination distance	1	<b>0.387</b>	0.387
	2	0.090	0.477
	3	<b>0.421</b>	0.898
Highest correlations with environmental variables	1	Se <sup>-2</sup> 0.432, Cu <sup>+2</sup> 0.319	
	2	Ra-226 <sup>+2</sup> 0.279, Ba <sup>+2</sup> 0.264	
	3	Temp. 0.558, HCO <sub>3</sub> <sup>-</sup> 0.430	



**Figure 3-10.** 2004 NMDS ordination of species abundance data. The lake groups do not cluster as tightly as in CCA (Figure 3-7) indicating that there are likely other environmental variables influencing the species composition and abundance within these lakes and pits. Many of the variables contained in AXIS2PCA, from the CCA environmental matrix, are correlated with axis 1 of this NMDS ordination.



**Figure 3-11.** 2004 NMDS biplot ordination of species plotted in species space. Species positioned similarly to lakes plotted in Fig. 3-10 are abundant in those lakes and are likely tolerant to the water quality within their associated lakes or pits.

## 2005

Group 4 lakes have similar species composition and abundance as they form a loose cluster on the positive side of axis 1 (Fig. 3-12). Gaertner Pit shows the greatest separation from the other group 4 members on both axis 1 and 2, indicating that its species composition and abundance is different from the other group 4 lakes.



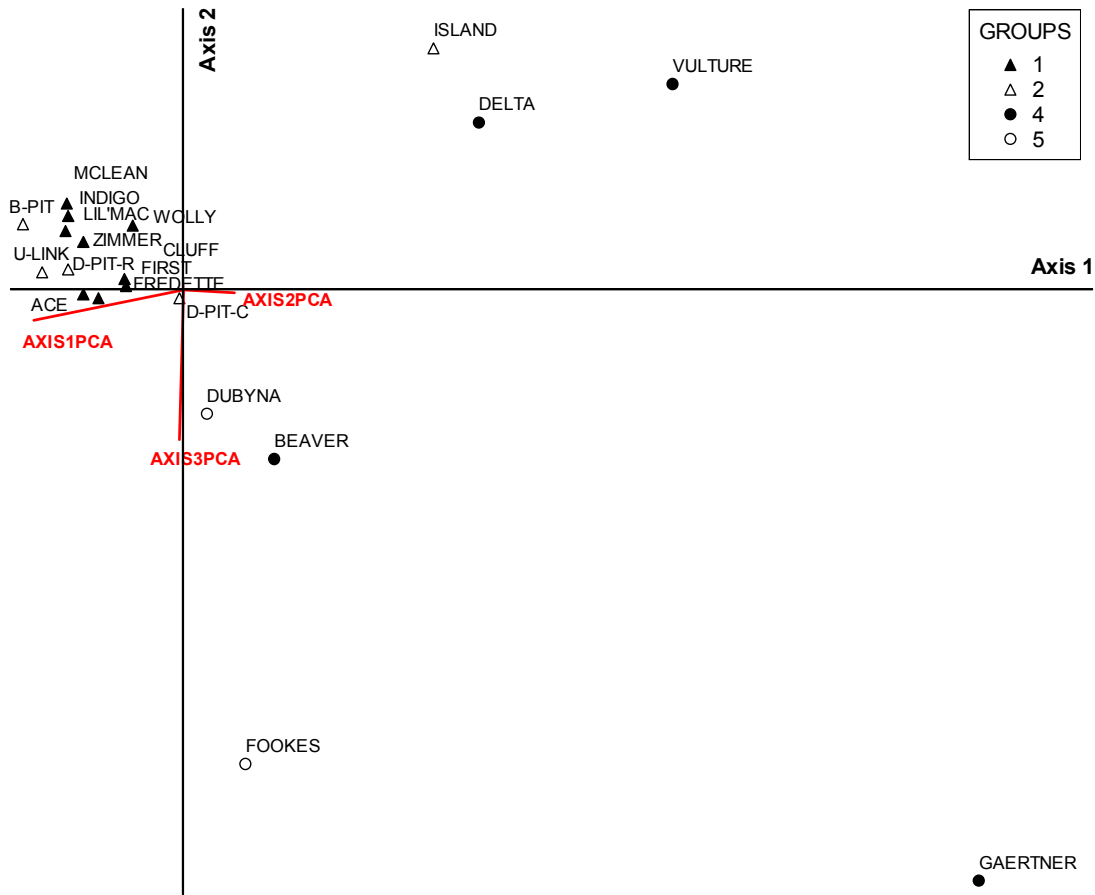
Island Lake is positioned close to Delta Lake and Vulture Lake (Fig. 3-12), meaning that Island Lake has similar species composition and abundance to these lakes. Island Lake was classified into lake group 4 in previous years, however, cluster analysis classified Island Lake into group 2 in 2005. This is likely due to a decreasing trend in TDS from 2003 to 2005 (see Table A1-1). All of these lakes are positioned on the right side of axis 1 due to their higher concentrations of  $\text{Al}^{+3}$ ,  $\text{Cu}^{+2}$ ,  $\text{Fe}^{+2}$  and  $\text{HCO}_3^-$  relative to the other lakes. It is these water quality variables that are associated with AXIS2PCA, which projects from the origin of the ordination towards the right side of axis 1 (Fig. 3-12).

Groups 1 and 2 have similar species composition and abundance in 2005 and these two groups (with the exception of Island Lake) form a tight cluster near the origin of the ordination. These lakes are positioned on the left side of axis 1 according to their higher concentrations of  $\text{B}^{+3}$ ,  $\text{Ca}^{+2}$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Mg}^{+2}$ ,  $\text{Na}^+$ ,  $\text{SO}_4^{-2}$ , and hardness relative to the other lakes. These water quality variables are associated with AXIS1PCA, which projects towards the left side of the ordination (Fig. 3-12).

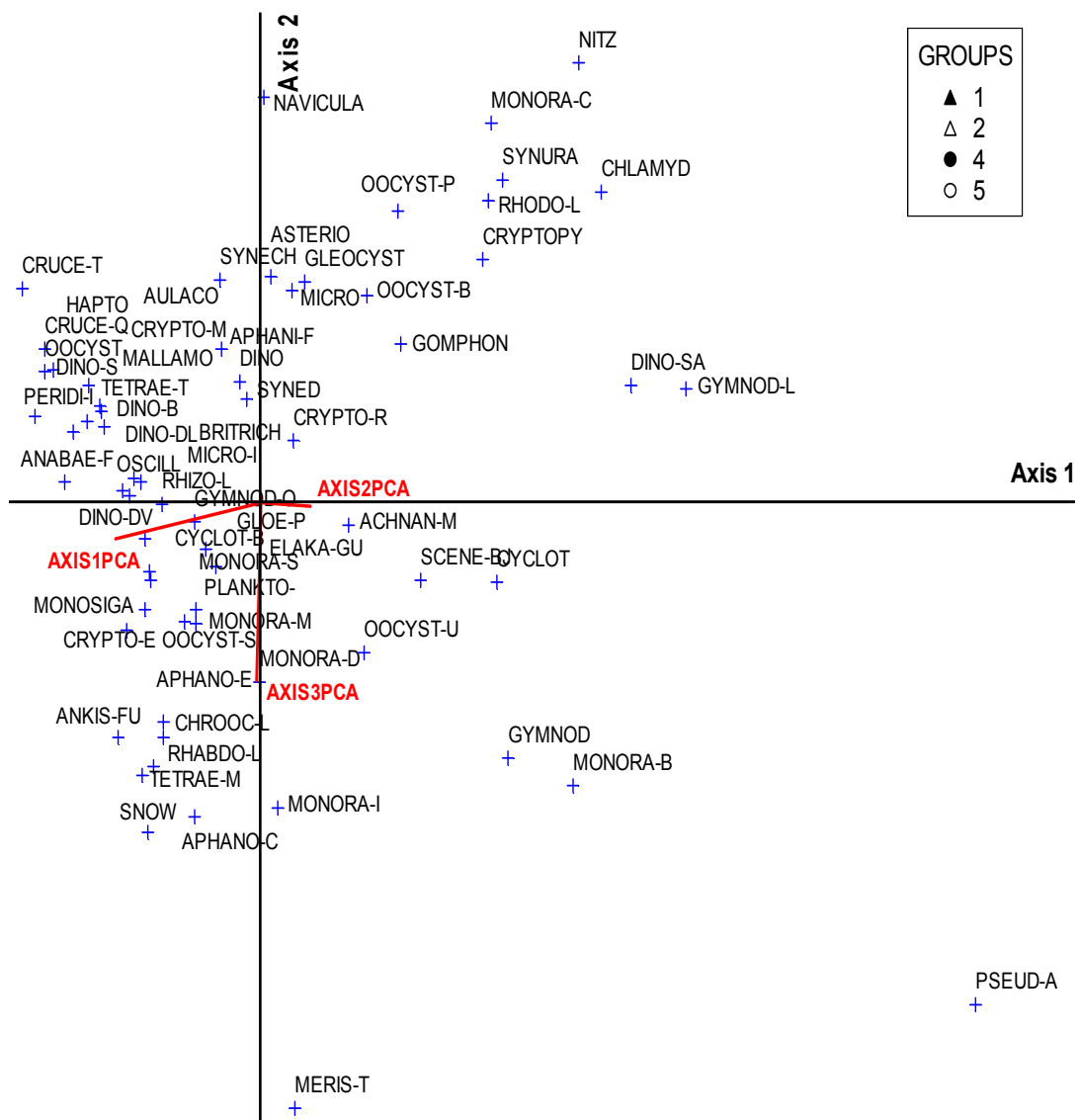
Species composition and abundance is similar among group 5 lakes, as indicated by their similar positioning on the ordination (Fig. 3-12). These lakes are aligned with AXIS3PCA, which is associated with  $\text{Ba}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{U}^{+3}$ , and  $\text{Ra-226}^{+2}$ .

*Merismopedia tenuissima* (Merist-T) is abundant in Fookes lake, while *Pseudanabaena arcuata* (Pseud-A) is associated with Gaertner Pit (Fig. 3-13). These species are positioned similarly to their respective lakes on the ordinations because they are tolerant to the water qualities of Fookes Lake and Gaertner Pit.

The percent variance explained by CCA is low, however there is a strong correlation (0.546) between raw data distance and ordination distance for axis 1 (Table 3-6). Only axis 1 shows a significant relationship between the species and environment matrices. Thus, only AXIS 1 was considered for interpretation.



**Figure 3-12.** 2005 CCA ordination biplot of lakes plotted in environmental space based on species composition and abundance data. Species composition and abundance is similar in lake groups 1 and 2 and cluster near the origin. Island lake is more similar to the group 4 lakes as indicated by its positioning near Vulture and Delta lakes. The water quality variables associated with AXIS1PCA and AXIS2PCA (Table 3-1) influence the positioning of lakes and pits along axis 1. The water quality variables associated with AXIS3PCA influence the positioning of lakes and pits along axis 2.



**Figure 3-13.** 2005 CCA biplot of species plotted in environmental space. Species positioned on the left side of the ordination are tolerant to the water quality variables associated with AXIS1PCA, while species positioned on the right side are tolerant to the water quality variables associated with AXIS2PCA (Table 3-1). Species that are positioned similarly to the lakes in Fig. 3-12 are tolerant to the water quality within their respective lakes.

**Table 3-6.** Summary of the results from the CCA of the 2005 species abundance data. Variance explained (%) is the highest for axis 1. Significant p-values associated with the Monte Carlo results indicate a strong relationship between the species data matrix and the environmental data matrix ( $\alpha = 0.05$ ) for AXIS 1. High correlations between raw-data distance and ordination distance indicate a good representation of the original data for axis 1. Biplot correlations show the strength of relationships between the environmental measurements and the ordination axes (representing the species data). Values that are ideal for interpretation are bolded and show that only axis 1 should be considered for interpretation.

	Axis	Increment	Cumulative	
Variance explained (%)	<b>1</b>	<b>8.4</b>	<b>8.4</b>	
	2	5.8	14.2	
	3	4.3	18.5	
Correlations between raw-data distance and ordination distance	<b>1</b>	<b>0.546</b>	<b>0.546</b>	
	2	-0.042	0.504	
	3	0.021	0.525	
Monte Carlo results	<b>1</b>	<b>0.018*</b>		
	2	0.098*		
	3	0.174*		
Biplot correlations		AXIS1PCA	AXIS2PCA	AXIS3PCA
	1	<b>-0.843</b>	0.284	-0.025
	2	-0.185	-0.014	-0.927
	3	0.505	0.959	-0.374

\*Values are p-values resulting from a Monte Carlo test.

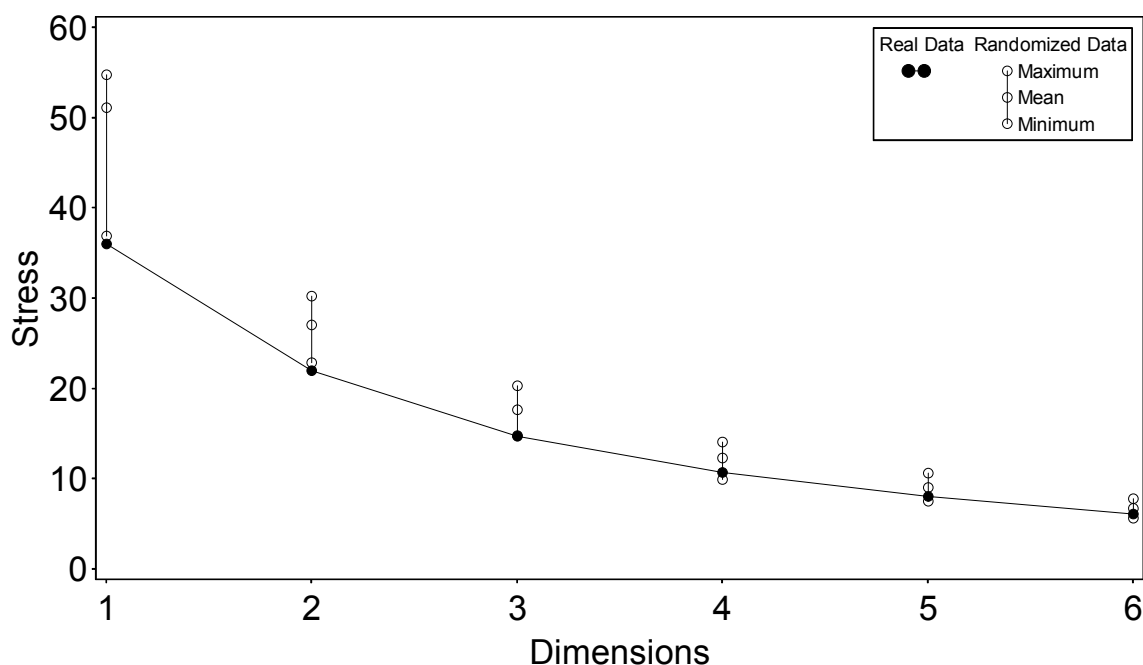
A two dimensional NMDS solution was appropriate for the 2005 species abundance and composition data due to the sharp decrease in stress that occurs over 2 dimensions (Fig. 3-14). Axis 1 is the most representative of the original data, as this axis has the highest correlations between raw data distance and ordination distance (Table 3-7). Thus, axis 1 is considered for interpretation. The environmental variables that had the highest correlations with axis 1 (sulphate and calcium) are similar to those associated with AXIS1PCA (CCA, Table 3-1). All 2005 lakes groups are significantly different from each other (MRPP,  $p = 0.002$ ,  $T = -3.19$ ,  $A = 0.164$ ). The similarities between NMDS and CCA suggest that the results are comparable.

The lake groups do not cluster as well in NMDS (Fig. 3-15) as in CCA (Fig. 3-12) supporting that there are environmental variables other than those measured during this study that are influencing the species composition and abundance in these lakes and pits. Lake and pits positioned along the left side of axis 1 of the ordination contain higher concentrations of  $\text{Ca}^{+2}$ , Hardness,  $\text{Ni}^{+2}$ ,  $\text{Se}^{-2}$ ,  $\text{SO}_4^{-2}$ , and  $\text{Zn}^{+2}$  relative to the other lakes and pits in the ordination, as indicated by the biplot vectors (Fig. 3-15 and 3-16). Lake and pits positioned along the right side of the ordination contain higher concentrations of As.

The species *Pseudanabaena arcuata* (Pseud-A) is associated with Gaertner Pit, while *Merismopedia tenuissima* (Meris-T), *Monoraphidium irregulare* (Monora-I) and *Oocystis solitaria* (Oocyst-S) are associated with Beaverlodge Lake. The lakes and their associated species are positioned similarly on the NMDS ordination (Fig. 3-15 and 3-16) indicating that these species are tolerant to the water quality within their respective lake or pit.

**Table 3-7.** Summary statistics from NMDS of 2005 species composition data. Axes 2 and 3 are more representative of the raw data as indicated by their higher correlations between raw data distance and ordination distance. The environmental variables that have the highest correlations with axes 2 and 3 are similar to those represented by PCAAXIS1 from CCA, meaning that NMDS and CCA results are comparable.

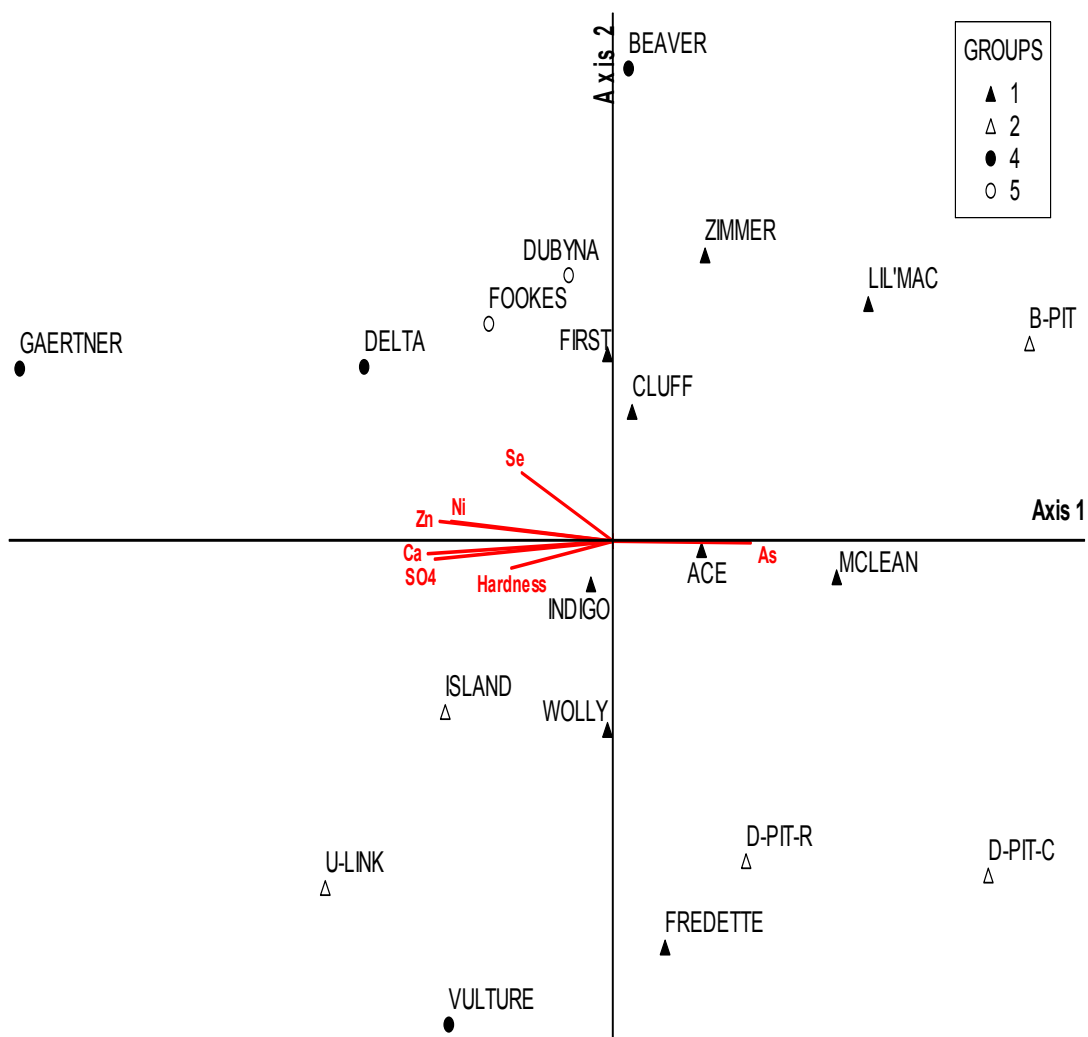
	Axis	Incremental	Cumulative
Correlations between raw data distance and ordination distance	1	0.456	0.456
	2	0.180	0.636
Highest correlations with environmental variables	1	$\text{Ca}^{+2}$ , 0.368, $\text{SO}_4^{-2}$ , 0.355, $\text{Zn}^{+2}$ , 0.345	
	2	$\text{B}^{+3}$ , 0.189, $\text{Fe}^{+2}$ , 0.162, $\text{Se}^{-2}$ , 0.088	



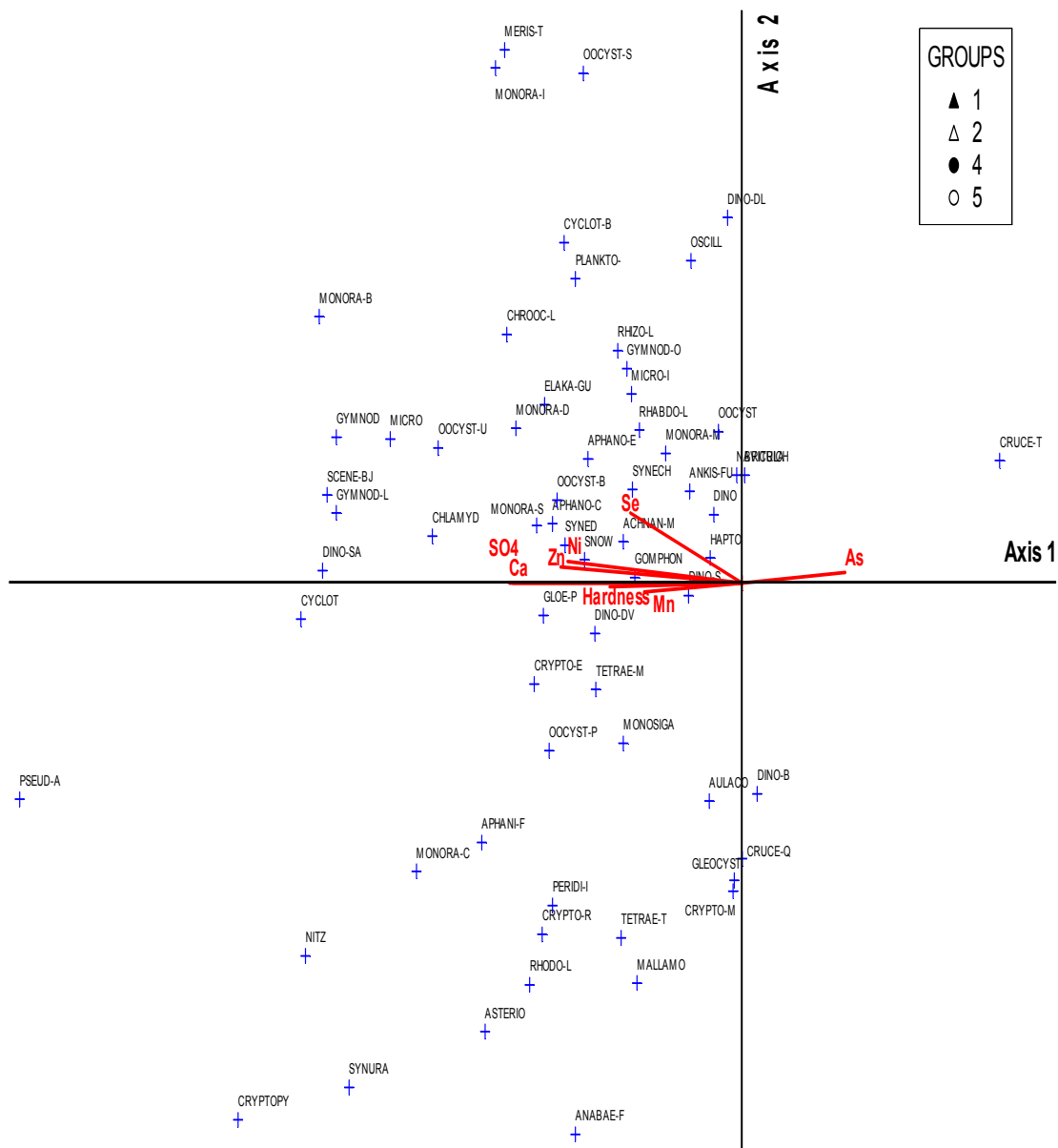
**Figure 3-14.** NMDS scree plot based on 2005 species abundance data. The sharp decrease in stress over the first two dimensions suggests that a 2-dimensional (axis) solution is appropriate for this data set. Plots for real data runs are below the means of the randomized runs for all recommended solutions and all real-run plots are significantly different from random-run plots ( $p \leq 0.05$ ).

### 3.3.3 Indicator Species Analysis

There are no instances where one particular species was representative of a group over multiple years. *Anabaena spiroides* was an indicator species in 2003 and 2004, but for different lake groups (Table 3-8). In 2003, there was at least one indicator species identified for each lake group. In 2004, there was only one lake from group 5 (Dubyna Lake) and Indicator Species Analysis requires that there be two lakes representing each lake group in order to perform the analysis. Therefore, Dubyna Lake was deleted from the matrix, resulting in the absence of indicator species for lake group 5. Results from 2005 reveal that there are indicator species for all groups except for lake group 2.



**Figure 3-15.** NMDS biplot of the 2005 species data showing lakes plotted in species space. Lake groups do not cluster as well as in CCA and there are likely other environmental variables that are influencing species composition and abundance in these lakes and pits. The vectors projecting from the origin show the environmental variables that best explain the positioning of the lakes along axes 1 and 2.



**Figure 3-16.** 2005 biplot ordination of species plotted in species space. Species positioned similarly to lakes plotted in Fig. 3-15 are likely tolerant to the water quality conditions in their respective lakes.



### **3.3.4 Proportional Distribution of Particulate Phosphorus Size Fractions in the Planktonic Food Webs**

The majority of lakes and pits contain all planktonic size fractions in their food webs (Fig. 3-17). However, there are a number of aquatic systems that have missing size fractions, or a strong dominance of certain groups of organisms. Lakes and pits that contain very different food web structure may be indicative of impacts from water quality. For example, B-pit, Sue-C (group 2), and Gaertner Pit (group 4) have no zooplankton >200  $\mu\text{m}$ . The 2 – 40 size fraction dominates the biomass within Sue-C Pit's planktonic food web, whereas the 0.8 – 40  $\mu\text{m}$  organisms dominate the planktonic biomass in Gaertner Pit.

Island Lake's food web structure appears to be unique within group 4 due to its higher proportion of biomass in the upper size fractions (2 – 200  $\mu\text{m}$ ) compared to Delta Lake and Vulture Lake. A potential relationship may exist between Island Lake's water chemistry (i.e. greater salinity than all other lakes and pits) and its unique food web structure.

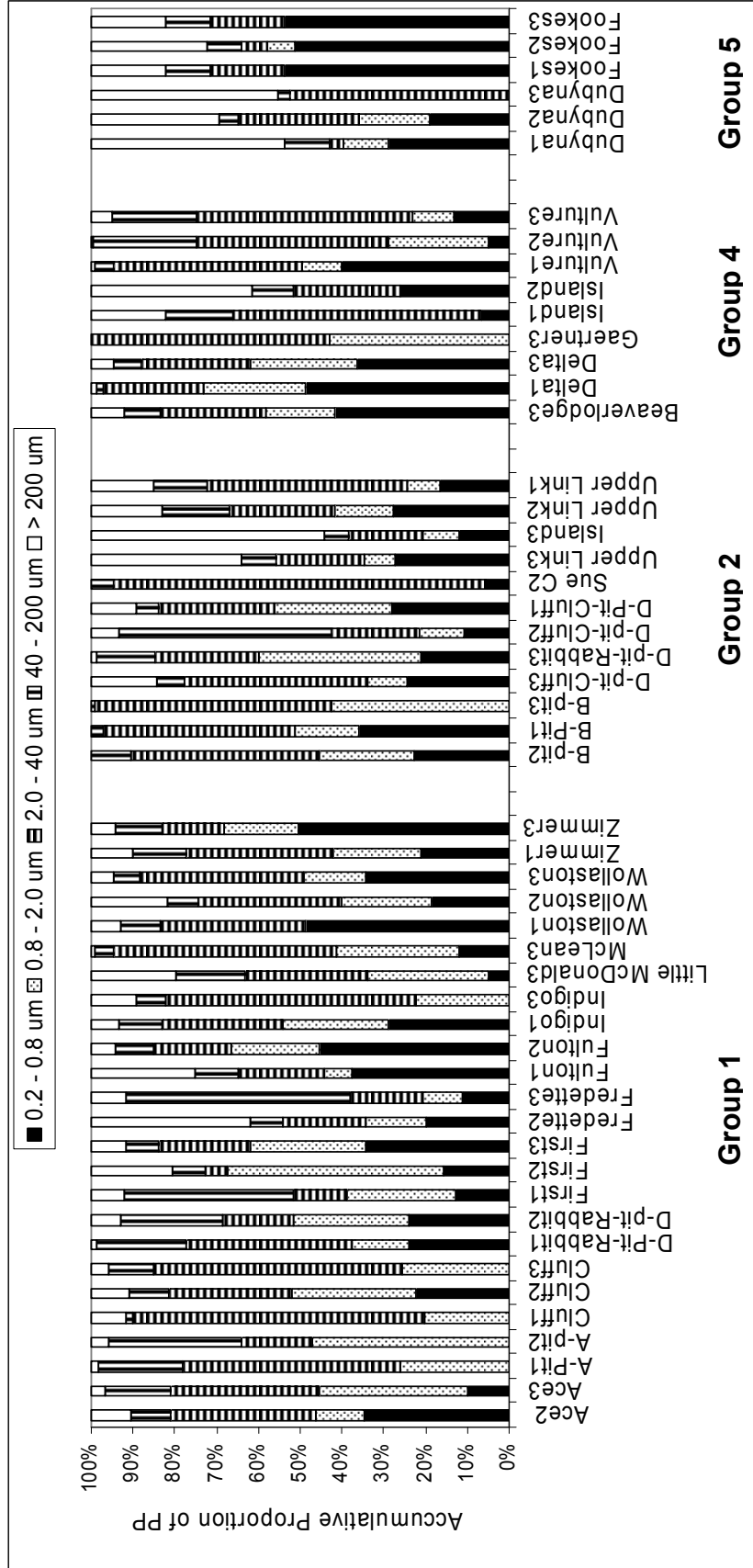
## **3.4 Discussion And Conclusions**

A direct relationship is evident between the water quality variables and plankton communities. The four lake groups also cluster when superimposed onto ordinations derived from plankton composition and abundance data. However, the relationships are variable as noted by the lack of tight clustering among some lakes in their respective groups in different years. This variance may be explained by examining the results in each year (also see Section 3.4.2). Furthermore, the low amount of variance explained by CCA indicates that there must be other environmental factors affecting plankton composition and abundance (also see Section 3.4.3).

**Table 3-8.** The significant indicator species and their associated lake groups for each sampling year. There was only one lake from group 5 (Dubyna Lake) in 2004. Therefore, Dubyna Lake was deleted from the matrix, resulting in the absence of indicator species for lake group 5 in that year.

Year	Group	Species Key	Actual Name	P-value
2003	1	ASTERIO	<i>Asterionella formosa</i>	0.009**
		GYNMOD2	<i>Gymnodinium</i> sp. 2	0.003**
		KEPHYR	<i>Kephyrion</i> spp.	0.036**
	2	DINO-D	<i>Dinobryon divergens</i>	0.049**
	4	NAVICULA	<i>Navicula</i> spp.	0.088*
	5	ANABAE – S	<i>Anabaena spiroides</i>	0.019**
		APHATH-N	<i>Aphanothece nidulans</i>	0.023**
		BRITRICH	<i>Bitrichia chodatii</i>	0.026**
		QUADRIG	<i>Quadrigula lacustris</i>	0.013**
		RHOPA	<i>Rhopalodia</i> spp.	0.009**
2004	1	ANABAE-S	<i>Anabaena spiroides</i>	0.005**
		KELLICOT	<i>Kellicottia</i>	0.021**
	2	NITZ	<i>Nitzschia</i> spp.	0.097*
	4	KERATELA	<i>Keratella</i>	0.055*
	5	MONORA	<i>Monoraphidium</i> spp.	0.037**
		Not enough lakes in this group for analysis		
2005	1	HAPTO	Haptophyceae	0.011**
	2	No significant indicator species		
	4	GYMNOD-L	<i>Gymnodinium pusillum</i>	0.057*
	5	APHANO-C	<i>Aphanothece clathrata</i>	0.002**
		CHROOC-L	<i>Chroococcus limneticus</i>	0.008**
		RHABDO-L	<i>Rhabdoderma lineare</i>	0.002**
		ELAKA-GU	<i>Elakatothrix genevensis</i>	0.02**
		MONORA-D	<i>Monoraphidiu dybowskii</i>	0.026**
		OOCYST-U	<i>Oocystis pusilla</i>	0.021**
		GYMNOD	<i>Gymnodinium</i> sp	0.046**

\*  $\alpha = 0.01$  \*\*  $\alpha = 0.05$



**Figure 3-17.** Distribution of biomass within the planktonic food webs. Biomass is represented as particulate phosphorus. Each size fraction represents a size range of planktonic organisms. Lake and pits with missing fractions, or a strong domination of a particular fraction, may be related to differences in water quality.

### **3.4.1 Similarity in species composition and abundance among data matrices**

Mantel test showed a significant association between the 2003 and 2004 species composition and abundance matrices, as well as between 2004 and 2005. In contrast, the 2005 and 2003 matrices were not significantly associated with respect to plankton composition and abundance. A possible reason for this is that two different phytoplankton identification companies were used over the three year sampling period. The 2003 and 2004 data matrices were identified using Algatax Consulting, while the 2005 matrix was identified using Bio-Limno Research and Consulting. However, the lake groups still clustered in an informative manner in the 2005 CCA ordination. Therefore, the relationships between plankton composition and water quality observed in this study are not likely due to differences in identification techniques.

### **3.4.2 Direct relationships between water quality, species composition, and species abundance for each sample year**

#### 2003

There are two lakes that are positioned at a distance from their respective group members in the CCA ordination (Fig. 3-1). These lakes are Island Lake (group 4) and Upper Link Lake (group 2). Island Lake is positioned at the far right of axis 1 and AXIS1PCA is the bi-plot vector that is correlated with axis 1 (Fig. 3-1). AXIS1PCA represents the water quality variables that are highly correlated with axis 1 of the ordination. The ordination is plotted in environmental space, rather than species space, therefore Island Lake is separated from its other group members. Within CCA, the species matrix is constrained by the environmental matrix (water quality variables) resulting in the water quality characteristics influencing the positions of the lakes in ordination space (He et al. 2007). However, the species matrix is also considered, and therefore, the separation of Island Lake from its other group members also reflects differences in species composition and abundance in response to the measured environmental variables.

An ordination plot of species in environmental space shows that species composition responds to the water quality variables (Bona et al. 2007). There are a few species of phytoplankton that are abundant in Island Lake (e.g. *Ankistodesmus falcatus*, *Chroococcus minutes* and *Tetraedron minimum*, Fig. 3-2). They have high correlations with axis 1 (0.480, 0.520 and 0.543, respectively) and have similar positioning to Island lake on the ordination. Many species of freshwater plankton occur in weakly saline lakes, such as Island Lake. This salinity is associated with the water quality variables represented by AXIS1PCA. The TDS in Island Lake was 2562 mg L<sup>-1</sup> rendering it an oligohaline lake (Marshall et al. 2006).

Recent studies have shown that both chlorophytes (*Ankistodesmus falcatus*, *Tetraedron minimum*) and Cyanobacteria (*Chroococcus minutes*) can tolerate a salinity gradient ranging from freshwater (<500 mg L<sup>-1</sup> TDS) to polyhaline (>18000 mg L<sup>-1</sup> TDS) (Marshall et al. 2006). Pilkaityte et al. (2004) found that certain phytoplankton species (Cyanobacteria) increased in biomass in response to increasing salt loads in a mesocosm study. These increases in biomass were accompanied by a shift in species composition. Island Lake may therefore be separated from Delta Lake and Vulture Lake (also in group 1) because of its greater salinity and associated phytoplankton species.

Diversity indices are often useful to compare similarities and differences among lakes (see Appendix 4). For example, Shannon's Diversity Index considers factors such as species richness and species evenness to obtain an index of diversity in a given lake (Sommer 1995). However, nothing unusual about Island Lake is immediately evident from these statistics. The biodiversity of Island Lake is very similar to other lakes in the data set, which provides supporting evidence for a well adapted and diverse phytoplankton community in Island Lake.

A more complete comparison of the whole planktonic food web structure (microbial to zooplankton) among the group 4 lakes can be seen using the accumulative proportions of particulate phosphorus across various size fractions (Fig. 3-17). Island Lake has an abundance of zooplankton (> 200 µm fraction) compared to the other two lakes. It is missing the 0.8 – 2.0 µm size fraction, which is not the case for Delta Lake and Vulture Lake. The literature states that certain species of

freshwater zooplankton can tolerate elevated salinity concentrations (Evans et al. 1996; Martinez-Jeronimo and Martinez-Jeronimo 2007; Mohammed and Agard 2007; Sarma et al. 2006), which may provide a competitive advantage to tolerant zooplankton over more sensitive taxa. Island Lake's TDS concentration of 2562 mg L<sup>-1</sup> is much greater than those of Delta Lake (480 mg L<sup>-1</sup>) and Vulture Lake (338 mg L<sup>-1</sup>). Derry et al. (2003) found that zooplankton could be found in lakes with TDS concentrations up to 3000 mg/L, where the dominant anions were either Cl<sup>-</sup> or SO<sub>4</sub><sup>-2</sup>. The dominant ion causing high salinity in Island Lake is SO<sub>4</sub><sup>-2</sup> (Table A1-1). It is common for saline lakes in Saskatchewan to exhibit high SO<sub>4</sub> (Evans, 1996), however the chemical characteristics of Island Lake are directly related to its exposure to treated effluent from mining activities (Cogema 2000), rather than natural sources.

A similar situation exists among the 2003 group 2 lakes, where Upper Link Lake is separated from its group members (B-pit and D-Pit Cluff) along axis 2 (Fig. 3-2). Although Axis 2 is difficult to interpret due to its low distance preserving properties, it does contain some useful information. For instance, *Euglena polymorpha* Dangeard, *Eudorina* sp. and *Chlamydomonas* spp. are positioned high on the axis, similar to Upper Link Lake in Fig. 3-1 and these species are abundant in Upper Link compared to other lakes (Table A3-2). It is not clear why these species are abundant in Upper Link Lake, as there are no water quality indicator variables that distinguish Upper Link from its group members. However barium, radium-226 and uranium (water quality variables that were not indicators of group 2) are elevated in Upper Link compared to the other two lakes (Table A1-1). Unicellular algae species, such as those abundant in Upper Link Lake, are tolerant to elevated concentrations of uranium (Dessouki et al. 2005), radium and barium (Szabo 1967). There is a strong dominance of organisms in the 2 – 40 µm size range (Fig. 3-17), which includes *Euglena polymorpha* Dangeard, *Eudorina* sp. and *Chlamydomonas* spp.

The reasons for Upper Link being unique are not as clear as for Island Lake, but one could speculate that the elevated levels of radionuclides (Ra-226<sup>+2</sup> and U<sup>+3</sup>) and Ba<sup>+2</sup> have given Upper Link Lake distinct water chemistry characteristics and

species composition and abundance. It is also important to remember that the amount of variance accounted for by CCA was low, compared to NMDS, indicating that there are other environmental factors that were not measured that may be influencing phytoplankton composition and abundance (also see Section 3.4.3).

There is a general pattern of lakes and pits with elevated metal concentrations to have unique food web structure relative to the majority of lakes and pits. However, Island Lake does not fit this pattern. Island Lake's food web structure is likely related to its salinity, rather than elevated metal concentrations.

#### 2004

Sue-C Pit has a very different food web structure compared to its other group members (Fig. 3-17). This is evident by Sue-C's isolated position in ordination analysis (Fig. 3-7). The >200 micron and 0.8 – 2.0 micron size fractions are missing, while the 2 – 40 micron size fraction dominates the biomass. Some possible reasons for these missing groups of plankton could be the amount of contamination in the pit or the recent event of flooding in 2002.

Sue-C Pit is contaminated heavily with arsenic ( $45 \mu\text{g L}^{-1}$ ), copper ( $17 \mu\text{g L}^{-1}$ ) and zinc ( $22 \mu\text{g L}^{-1}$ ). These metals are toxic to aquatic organisms (Hjorth et al. 2006; Le Jeune et al. 2006). Hjorth et al. (2006) conducted a study on the effects of elevated zinc concentrations on a marine aquatic community and found that the phytoplankton community shifted in response to elevated zinc concentrations, resulting in a dominance of more tolerant species as sensitive species became extinct. Phytoplankton community shifts are common in response to changes in environmental conditions, including elevated concentrations of metals (Loez et al. 1995). The *Chlamydomonas* spp and *Geitlerinema* spp. appear to be tolerant to the water quality of Sue-C Pit, as these species are abundant in the pit (Fig. 3-8). However, the positioning of these species is also being influenced by AXIS1PCA, which is also aligned with Island Lake on the ordination (Fig. 3-8). Thus, the *Chlamydomonas* spp and *Geitlerinema* spp. are also tolerant to Island Lake's water quality.

Other studies have reported the sensitivity of zooplankton species to certain metals. Vinot and Pihan (2005) cited significant decreases in zooplankton biomass associated with copper concentrations as low as  $20 \mu\text{g L}^{-1}$ . This study also reported that a decrease in planktonic abundance and dominance of tolerant species was noted at copper concentrations of  $27 \mu\text{g L}^{-1}$ . This may explain the missing size fractions and dominance of other size fractions in some pit lakes (i.e. Sue-C pit and B-pit).

Another factor that may affect the plankton food web structure of Sue-C Pit is the short temporal scale for community colonization and the way the pit was flooded. Sue-C Pit was flooded in 2002 and the planktonic food web may not have had adequate time to develop before sampling (2004). In contrast, D-pit at Cluff Lake has been flooded since 1983 and has had more than two decades of community development and has developed a planktonic food web that is similar to those of the reference lakes. It is possible that Sue-C may naturally remediate over time, as D-pit has.

Additionally, Sue-C Pit was flooded by precipitation and groundwater inflows. There are no known surface water inflows into Sue-C Pit other than local drainage. The establishment of plankton would likely be slow under these conditions because little biota would be recruited from surface runoff. Dispersal and recruitment of plankton from nearby water bodies may be restricted to wind and other forms of physical transport (e.g. waterfowl, boats, humans).

## 2005

A large core of lakes is grouped tightly near the origin in the 2005 CCA ordination (Fig. 3-12), with only a few that are distant from this group. Lake group 2 is nested within the cluster near the origin of Fig. 3-12 with the exception of Island Lake. In 2003 and 2004, Island Lake was in lake group 4 (along with the other effluent receiving lakes). The reason for this is a substantial improvement in water quality from 2003 to 2005. Improved water quality is noted by a drop in total dissolved solids from  $2562 \text{ mgL}^{-1}$  in 2003 to  $1294 \text{ mgL}^{-1}$  in 2005. Decreased TDS



can be attributed to the cessation of effluent discharge into Island Lake in 2002 and subsequent natural flushing by the surrounding watershed.

Although Island Lake belongs to a different water quality group, it is positioned similarly to Delta Lake and Vulture Lake (effluent receiving lakes) on the CCA ordination. Island Lake is more similar to Vulture and Delta Lakes than to the other group 2 lakes (Appendix 2). Thus, many of the same species are abundant in these lakes (Fig. 3-13). For example, *Chlamydomonas spp.* and *Nitzschia spp.* were particularly common in all effluent receiving lakes across all years.

All group 4 lakes are positively loaded on axis 1 of the CCA ordination (Fig. 3-12). Gaertner Pit is the most solitary member of this group and is worthy of further discussion. Similar to Sue-C Pit, Gaertner Pit is another recently flooded pit that contains a host of elevated contaminants ( $\text{Ni}^{+2}$ ,  $\text{Ra-226}^{+2}$ ,  $\text{U}^{+3}$ ,  $\text{Se}^{-2}$ ) relative to most other lakes and flooded pits in the data set (Table A1-1). Two of these variables ( $\text{U}^{+3}$  and  $\text{Ra-226}^{+2}$ ) are captured by AXIS3PCA (Table 3-1), which is highly correlated with Axis 2 in the 2005 CCA ordination and explains why Gaertner Pit is positioned at the negative extreme of axis two. Gaertner Pit also has low concentrations of the major ions associated with AXIS1PCA, which explains its positioning at the extreme right side of Axis 1 in the ordination.

Summary statistics (Appendix 4) indicate that Gaertner Pit is populated by only 2 species and has the lowest Shannon's Diversity Index of all water bodies in the data set. The most abundant species showing tolerance to the pit conditions is Cyanobacterium *Pseudanabaena arcuata* (PSEUD-A) and occupies a similar ordination position to the pit (Fig. 3-13). Certain species of Cyanobacteria are known to be tolerant to high concentrations of metals. For example, Storni et al (2007) stated that some species of Cyanobacteria have nickel-processing systems that make use of nickel-based enzymes allowing them to tolerate nickel concentrations up to  $58 \text{ mg L}^{-1}$ .

The planktonic food web structure of Gaertner Pit is also distinct from the other group 4 lakes (Fig. 3-17). Similar to Sue-C Pit, Gaertner has missing size fractions, most notably the complete absence of zooplankton ( $>200 \text{ }\mu\text{m}$ ) and the domination of organisms from  $0.8 - 40 \text{ }\mu\text{m}$ . These characteristics follow the

predictions of Odum (1985), who stated that stressed ecosystems often select for small bodied organisms and select against larger, more sensitive organisms (i.e. large zooplankton). Gaertner Pit provides another example of a recently flooded contaminated pit that exhibit poor biodiversity and a profoundly different food web structure compared to other group 4 lakes and to most other study lakes.

### **3.4.3 General Relationships between Water Quality, Species Composition and Species Abundance**

There are direct relationships between water quality and species composition and abundance, which are most prominent in lakes and pits that have received a high degree of exposure to mining activities . Across all sample years, CCA positioned several lakes at the extremes of the ordination axes, which indicates that their water quality and species composition are unique in these systems compared to other lakes in the data set. These results also indicate that lakes with the greatest concentrations of contaminants have the lowest biodiversity (i.e. low species richness in Sue-C Pit and Gaertner Pit, Appendix 4) and distinct differences in species composition and food web size structure (i.e. Island Lake, Upper Link Lake, Fookes Lake) when compared to less exposed (or non-exposed) lakes in the data set. Sue C Pit1 (2003) was removed from the 2003 species matrix because it was identified as an outlier. However, the 2003 sample of Sue-C pit was very similar to the 2004 sample. Particularly, in 2003 Sue-C Pit also had a strong domination of the 2 – 40 size fraction and only 3 species could be identified in the pit. The differences in species richness and composition that were observed among lake groups, are tied to a number of theories in the literature involving the effects of multiple stressors on biodiversity and community structure.

The effects of multiple stressors is unknown and difficult to predict in aquatic environments due to the number of biotic and abiotic factors that may cause synergistic or antagonistic cumulative effects on species richness, species composition and abundance, and community size structure (Antunes et al. 2007; Christensen et al. 2006; Folt et al. 1999). Vinebrook et al. (2004) describes hypothetically how responses of species to multiple stressors may lead to community

tolerance or community sensitivity. If an aquatic community contains several species that are tolerant to the surrounding contaminants, then stress-induced community tolerance, or pollution-induced community tolerance (PICT, Blanck and Wangberg 1988) can result. This would exemplify an antagonistic effect, as the cumulative effect of exposure to multiple stressors is less than what would be expected from the additive effects of the stressors. This antagonistic effect is due to community resilience.

Conversely, if many species are sensitive to the surrounding water quality, then few, if any, species will survive. This would be an example of stress-induced community sensitivity. In this case, the cumulative effect of multiple stressors would be additive, or synergistic, and the resulting aquatic community would be extremely fragile.

It seems that both antagonistic and synergistic effects of water contaminants are present when looking at the most highly exposed lakes in this study. For example, Island Lake is consistently identified on CCA, and to a lesser extent on NMDS, ordinations as being separated from the core group of non-impacted lakes. However, the average species richness within Island Lake was 14 (range of 13 – 16 for all three sample years) and it had a food web structure similar to the reference lakes (Fig. 3-17). Given that Island Lake experienced a 28 fold increase in salinity over baseline conditions from the release of effluent into the lake, the planktonic community contains an assortment of tolerant species that have been present over the temporal scale of this study. The cumulative effects of environmental stressors in Island Lake (namely increased concentrations of salts and some metals) are antagonistic. The planktonic species composition and abundance is very different from surrounding lakes, yet the food web structure and species richness are similar to less exposed lakes and pits.

The plankton food webs of Sue-C Pit and Gaertner Pit may represent the alternate theory of stress-induced community sensitivity. The heavy contamination by multiple stressors and extremely low species richness and diversity in these aquatic systems implies that very few planktonic organisms are tolerant to the pit conditions. As a result, these pits are isolated in the CCA ordinations and show

direct relationships between water quality, species composition and species abundance. However, the age of these pit-lakes may also be an important factor influencing the planktonic food web development. More long-term sampling of these pits is needed to determine if their planktonic communities will remain limited due to chemical stress, or become more diverse and tolerant through the addition of other resistant species. Resistant communities have enough genetic diversity among different species (or among individuals within the same species) to adapt to the surrounding environment, thus becoming more tolerant to stressed environmental conditions over time (Chapin et al. 1993).

Another common characteristic of Sue-C and Gaertner pits is that both contain a large proportion of small bodied phytoplankton, especially in the 2 – 40  $\mu\text{m}$  range. The presence of plankton in highly contaminated environments is well documented (Odum 1985). Smaller organisms are often more tolerant to contamination (Kalin et al. 2001; Vinebrook et al. 2004) due to their short-lived, fast reproducing life cycles. These characteristics allow such organisms to adjust to the surrounding water quality due to genetic mutation or acclimation (Cattaneo et al. 1998). In addition to life cycle characteristics, the greater metabolic rate of smaller organisms is also considered an advantage to tolerating contaminants (Chappell 1992; Fenchel 1974). Organisms with faster metabolism are able to purge contaminants out of their bodies more rapidly, thus reducing the toxic effect of contaminants (Cattaneo et al. 1998; Fenchel 1974).

The results of this study support the hypothesis that water containing elevated concentrations of metals and salts influences plankton community dynamics in lakes. Further support is shown by the results of the Multi-Response Permutation Procedures (MRPP). All lake groups within each sample year had significantly different phytoplankton communities at the  $\alpha = 0.10$  level. Results for 2003 and 2005 were significant at the  $\alpha = 0.05$  level ( $p \leq 0.08$  for 2004). However, the environmental variables used in this study are not the only factors that influence phytoplankton community ecology. For example, the majority of less impacted lakes in the data set consistently clustered near the origin of the CCA ordinations indicating that there is

little to no relationship between the measured environmental variables and the species data for these aquatic systems.

In all sample years, NMDS ordinations revealed much less clustering of lake groups than CCA ordinations. NMDS is an indirect gradient analysis (McCune and Grace 2002); it does not constrain the species matrix with an environmental matrix. The environmental matrix is analysed separately and the biplot vectors, associated with the environmental variables, portray indirect relationships with the species matrix. NMDS broadens the scope of influential factors affecting the plankton food webs of these lakes and pits, as indicated by less distinct clustering of lakes groups in the ordinations compared to CCA. Nonetheless, CCA and NMDS ordinations were broadly comparable, thereby increasing the confidence in the results.

Group 1 lakes possess good water quality due to no, or low, exposure to mining activities and consistently clustered around the origin of the CCA ordinations in all sample years. Group 1 lakes represent the core of lakes showing no impact from mining. Other lakes and pits that are positioned near group 1 on the ordinations also show little impact from mining exposure, regardless of the variety of exposure mechanisms identified for these lakes (Chapter 2). McClean Lake was identified as an outlier in the 2004 species matrix. The reason for this is because McClean Lake has a large number of abundant species and few rare species, relative to the other lakes and pits sampled in 2004. As a result, it was necessary to remove McClean Lake from the 2004 species matrix to remain consistent in our data manipulations across all sample years.

There are many complex biotic and abiotic variables that influence plankton food web dynamics in temperate lakes (Kalff 2002; Wetzel 1983). Some of these variables include latitude, trophic status, seasonal mixing and lake stratification patterns. When lakes of similar latitude are compared, differences in physical characteristics of the lakes and their associated drainage basins become important (Kalff, 2002). Furthermore, interactions among aquatic biota such as competition for nutrients among phytoplankton (Domingues et al. 2005), as well as differences in grazing pressure by zooplankton and fish (Carpenter et al. 1985; Larocque et al. 1996; McCauley and Kalff 1981; Venable et al. 2007) are often similar among

aquatic systems in the same region and season. The majority of lakes and pits in this study can be considered temperate, dimictic lakes due to their seasonal mixing patterns and latitude. As a result, there are similar physical (e.g. latitude, light penetration, seasonal mixing and stratification patterns) and biotic factors (competition for nutrients) that influence the seasonal succession of plankton in all study systems. Therefore, several non-impacted lakes are consistently clustered around the origin of the CCA ordinations because the planktonic food webs are not as affected by contaminants as they are by natural factors. The ordination positions of such lakes are likely better explained by environmental factors that typically regulate spatial and temporal variation in plankton species composition and abundance.

The objective of this chapter was to search for causal relationships between water quality and species composition and abundance. Such relationships were found, indicating that there are associations between exposure to mining activities and the composition and abundance of planktonic species within these study systems. Species richness and planktonic food web structure also appear to be influenced by high concentrations of metals and salts. The effect that these differences in community composition, abundance and food web structure have on ecosystem function (e.g. biogeochemical cycling and planktonic respiration) is the topic of chapter 4.

## **CHAPTER 4. RELATIONSHIPS BETWEEN WATER QUALITY, BIODIVERSITY AND ECOSYSTEM FUNCTION**

### **4.1 Introduction**

The terms biodiversity, ecosystem function, and anthropogenic stress allude to a growing area of research (Loreau 2000; Loreau 2004). Historically, there has been much debate about the relationships between biodiversity and ecosystem function (Duffy et al. 2007; Mooney 2002), biodiversity and ecosystem stability (Tilman et al. 2006b), the effects of anthropogenic stress on biodiversity (Relyea and Hoverman 2006), and the response of ecosystem properties (i.e. functioning) to changes in environmental conditions (i.e. chemical stresses) and species diversity (Balvanera et al. 2006).

The term biodiversity has many meanings depending on the context in which it is used in the literature. Some of the terms used to describe biodiversity include species richness (the number of species present), species evenness (how even the distribution is among species), species composition (which species are present), functional diversity (the abundance of species groups that perform different ecosystem-level functions), community diversity (abundance and spatial distribution of communities; also referred to as patchiness) (Walker 1992). Due to the large number of terms associated with biodiversity, it is critical for researchers to identify which definition they are using.

Accompanying the variety of terms associated with biodiversity is an equally daunting number of hypotheses, or theories to predict and explain the relationships between ecosystem function and biodiversity in response to external disturbances. Three important theories are the functional redundancy theory (Loreau 2004), the rivet-popper theory (Ehrlich 1991; Schiel 2006) and the idiosyncratic theory (Loreau et al. 2002).

Functional redundancy implies that the loss of species is not important because there are a number of alternate species that have similar roles in an ecosystem to replace those that are lost (Fonseca and Ganade 2001; Loreau 2004; Schiel 2006). Closely related to functional redundancy is the insurance hypothesis, which states

that increased biodiversity (namely species richness and trait diversity) decreases variability in ecosystem processes in response to environmental stress because there will be species present that can adapt or compensate for losses of sensitive species (Yachi and Loreau 1999). These two hypotheses seem contradictory at first in that redundancy seems to imply that biodiversity is not important, whereas the insurance hypothesis maintains that biodiversity is crucial. In a sense, redundancy theory has been used in the literature to question the importance of biodiversity, but only in questioning the minimum biodiversity required for the continued functioning of ecosystems (Lawton and Brown 1994). A merger of these two concepts is apparent in Naeem and Li (1997), who declared that larger numbers of species (richness) led to greater redundancy within functional groups, providing insurance that ecosystems will continue to function efficiently in the event of random species loss. In such a scenario, biodiversity becomes important because the resulting species redundancy provides the insurance that ecosystem function can resist change due to environmental stress. Conversely, it could also be argued that functional redundancy is ambiguous because of the multiple levels it can be addressed at: species level, population level and trophic level. Functional redundancy may also be explained by other principles such as niche differentiation and resource partitioning, which allow the coexistence of functionally similar species (Loreau 2004).

Opposing functional redundancy is the rivet-popper hypothesis. This concept was first proposed by Paul and Anne Ehrlich in 1981 (Loreau et al. 2002) and stated that species are analogous to rivets in an airplane. As each rivet is popped (species lost) the structure slowly weakens until the entire structure collapses (Schiel 2006). This implies that species are singular and each contributes to ecosystem function. Each species lost leads to a decline in maximum ecosystem function and therefore calls for the retention of all species in an ecosystem (Schwartz et al. 2000).

Both the functional redundancy and the rivet-popper hypotheses are assumed to have a monotonic (smooth) relationship between biodiversity and ecosystem function. The effects of species loss on ecosystem function may be cumulative (rivet-popper) or asymptotic (functional redundancy). Another proposed theory is of an idiosyncratic nature (Naeem et al. 2002). Naeem et al. (2002) explain that



additions or losses of species can have variable effects on ecosystem function leading to the idiosyncratic relationship between biodiversity and ecosystem function. This theory is commonly misinterpreted as showing no relationship between biodiversity and function due to the amount of variability and no clear trend. However, it has been demonstrated that idiosyncratic patterns can show predictable trends (Emmerson et al. 2001). In a mesocosm experiment looking at the effects of invertebrate diversity on ecosystem function (nutrient flux to the overlying water column), Emmerson et al. (2001) found that individual species treatments had different and variable effects on ecosystem function. Despite the variable (idiosyncratic) differences due to species composition, there was an overall increasing trend in the relationship between species richness and ecosystem function (nutrient flux). These results may not be surprising given that the maximum species richness was 4 in the highest treatment and all species used in the experiment were known to be dominant species in their natural environments. This would be similar to adding or removing a keystone species to an already low-diversity system, which would be expected to have highly variable effects on function. It is generally accepted that an idiosyncratic (highly variable) diversity-function relationship can be expected when biodiversity is extremely low (Naeem et al. 2002).

Many competing biodiversity-ecosystem function theories exist and determining which theories are pertinent depends on the current condition of the community or ecosystem of interest. For example, a system that is rich in diversity may follow the redundancy hypothesis due to an increased chance of there being compensatory species to replace those lost. One may predict that such a system would be more resistant to functional changes due to environmental stressors. Or, if the rivet hypothesis holds true, one might see a linear decrease in functioning as biodiversity decreases. On the other hand, an ecosystem with low diversity may not have adequate redundancy to buffer against stress. This may cause a highly variable (idiosyncratic) functional response.

It may be simpler to consider the effects of biodiversity on ecosystem function as either linear or asymptotic and observed trends may be monotonic or variable. Schwartz et al. (2000) reported that the expected relationship between biodiversity

and ecosystem function was asymptotic. The argument being that ecosystem function maximizes at some level of species richness that is below the species richness present in natural communities. This statement suggests that natural communities are primarily redundant and ecosystem function is expected to remain stable until a significant number of species go extinct, at which point entire functional groups could be lost (loss of functional diversity) and ecosystem functioning could be negatively affected. A reduction in predictability or a reduction in a process (e.g. biogeochemical cycling) would be interpreted as a negative affect on ecosystem function.

In this chapter, relationships are sought between biodiversity, ecosystem function and environmental stress (lake water chemistry) in planktonic food webs within northern temperate lakes exposed to uranium mining activities. In this study, biodiversity refers to species richness, species evenness, species composition and species abundance. To avoid confusion, I will specify which one of these terms is under consideration in each analysis. The ecosystem functions analysed are phosphorus cycling within the planktonic food web and planktonic respiration (dark-bottle oxygen depletion over time). The degree and the types of environmental stress caused by exposure to mining activities were dealt with in Chapter 2. The effects of environmental stresses and exposure mechanisms on species composition and abundance were evaluated in Chapter 3. The objective of the current chapter is to find relationships between water chemistry, biodiversity and ecosystem function.

## **4.2 Methods**

Please see Section 3.2.1 for field sampling methods. Subsequent processing is explained sub-sections below.

### **4.2.1 Planktonic Phosphorus Cycling**

#### Uptake

Four litres of lake water was sub sampled from each 40 L lake or pit sample and added to clear polyethylene containers that had been washed (0.1% Contrad-70<sup>®</sup>), rinsed (ethanol), and leached (0.1 N HCl). Carrier-free radiophosphate ( $^{33}\text{PO}_4^{-3}$ ,

130-21,000 Bq·ml<sup>-1</sup>, ICN Biomedicals) was added to each container, and then the container was sealed and gently shaken. Lake water was incubated in environmental chambers under fluorescent and incandescent light (~100-200 µmol m<sup>-2</sup> s<sup>-1</sup>, 15:9 L/D) at ambient temperatures (± 1 °C) which had a range of approximately 10 to 22 °C.

Planktonic uptake of radiophosphate was monitored for approximately the first 15 min. from injection. The change in dissolved radiophosphorus over time (e.g., at 1, 3, 5, 8, and 12 min) was measured with syringe filtration (5 to 10 ml, 25 mm diameter polysulphone, 0.2 µm pore size, Lida) (Hudson and Taylor 2005). Aliquots of unfiltered water (4 ml) were taken to determine total radioactivity in each incubation. Radioactivity was measured by liquid scintillation counting (Ecolume<sup>®</sup> counting fluid) and corrected for background radioactivity.

Radioactivity remaining in the dissolved fraction (i.e., total counts per minute in filtrate) over time was fitted to a polynomial function (Bentzen and Taylor 1991; Currie and Kalff 1984). The polynomial of best fit (by eye) to the initial time series points (e.g., at times equal to 0, 1, 2, and 5 minutes) was used. The uptake constant (k) was determined by taking the derivative of the polynomial at time zero and dividing by the mean radioactivity in the unfiltered samples (Currie and Kalff, 1984; Bentzen and Taylor, 1991). The reciprocal of this uptake constant is equal to the turnover time of the dissolved PO<sub>4</sub><sup>-3</sup> pool.

### Regeneration

The incubations (above) were continued for approximately 24 h to label the planktonic community. Incubations were terminated with the addition of unlabelled <sup>31</sup>PO<sub>4</sub><sup>-3</sup> as a competitive inhibitor (final concentration 1-5 mg L<sup>-1</sup>). This prevented re-incorporation of <sup>33</sup>P into microorganisms. Therefore, as <sup>33</sup>P was released from the plankton, it accumulated in the dissolved pool. The accumulation of <sup>33</sup>P in the dissolved pool was assayed (syringe filtration, 25 mm diameter polysulphone 0.2 µm pore size filters, Lida) at approximately one hour after competitive inhibitor addition and terminated approximately 30 h later. The slope resulting from the accumulation of dissolved <sup>33</sup>P over time provided an estimate of the release rate of dissolved <sup>33</sup>P.

The remaining lake water was analyzed for total phosphorus (TP), which was calculated as the sum of dissolved and particulate P (Parsons et al. 1984). Particulate P was analyzed in a similar manner to that outlined in (Hudson et al. 2001). Briefly, quantities of P in small particulate fractions (0.2-0.8, 0.8- 2 and 2-40  $\mu\text{m}$ ) were determined with syringe filtration with polycarbonate filters. Larger particulate fractions (40-200 and  $>200 \mu\text{m}$ ) were determined with nylon screens with a serial filtration approach. One to twenty litres of lake water were filtered in the serial filtration step to obtain a more precise estimate of P in these larger particulate fractions. In summary, TP was the sum of P in all volume corrected fractions plus dissolved P. Once an estimate of TP was obtained, the release rate (R) of dissolved P from the plankton was calculated with the following equation from Hudson and Taylor (1996):  $R \text{ (pM min}^{-1}\text{)} = (^{33}\text{P released (cpm L}^{-1}\text{)} \times \text{TP (pM)}) \div (\text{Total } ^{33}\text{P added to incubation (cpm L}^{-1}\text{)})$ . The abbreviation cpm represents radioactive counts per minute. Phosphorus regeneration is defined as the transfer of phosphorus from the particulate pool ( $>0.2 \mu\text{m}$ ) to the dissolved pool ( $<0.2 \mu\text{m}$ ) over time. Egestion, excretion, decay, cell lysis, cellular exudate, and sloppy feeding (un-ingested food) and other processes contribute to the dissolved pool. A full discussion of the assumptions and tests of the regeneration technique are provided in Hudson and Taylor (1996).

In addition to the regeneration rate, the turnover rate of planktonic P was also calculated by dividing the regeneration rate ( $\text{nM day}^{-1}$ ) by total particulate phosphorus (nM) to estimate the turnover rate of the particulate P pool (planktonic biomass, % per day).

#### **4.2.2 Total Planktonic Respiration**

Planktonic community respiration was determined by monitoring changes in dissolved oxygen. Lake water was sealed and incubated in dark BOD bottles (310 ml) containing sub-samples from our 40 L water collections. Oxygen microelectrodes (Unisense Corporation, Denmark) with a sensitivity of  $0.1 \mu\text{M}$  ( $3.2 \mu\text{g L}^{-1}$ ) were used to measure initial and final dissolved oxygen concentrations over a 48 hour incubation period. An incubation of 48 hours was used to detect low rates of oxygen

consumption in our oligotrophic lakes and pits. Incubations were carried out in environmental chambers at ambient temperatures ( $\pm 1$  °C) over a temperature range of approximately 10 to 22 °C.

Three sub-samples were taken from each 40 L lake or pit sample. Deionised water in a BOD bottle (identical to lake samples) was used as both a control and a calibration solution for our microsensors. The observed changes in dissolved oxygen over time in sample bottles were calculated as the difference between the control and lake or pit samples. Using deionised water as a control ensured that any changes in dissolved oxygen (DO) from slight changes in temperature and pressure would be accounted for over the course of the incubation. To ensure that the control maintained constant dissolved oxygen (zero oxygen consumption over the incubation period), a second deionised water sample (not used to calibrate the sensor) was monitored over the same incubation period. After calibration of the sensor, if both deionised water samples showed the same dissolved oxygen value, then the control was assumed to have not consumed oxygen due to bacterial contamination. After initial and final dissolved oxygen readings, oxygen consumption values from the sub-samples were corrected for changes in temperature and pressure by subtracting oxygen consumption values observed in the control. Subsequently, these values were volume corrected and reported as DO consumption in  $\mu\text{g L}^{-1} \text{d}^{-1}$ .

### **4.2.3 Multivariate Statistical Analysis**

#### **4.2.3.1 Principal Components Analysis (PCA)**

Correlation PCA (also see Chapter 3) was used to address two questions. Are there relationships between water quality and ecosystem function? And are there relationships between biodiversity and ecosystem function?

In order to examine the relationships between ecosystem function, water quality and biodiversity, the main data matrix consisted of phosphorus cycling variables for all years and respiration (2005 only) for the lake and pit samples. Phosphorus cycling data was available for all sample years and was merged to form a matrix containing columns of turnover time of the phosphate pool, planktonic regeneration rate and turnover rate of particulate phosphorus, while the rows

consisted of all lake and pit samples from all years. Sub-matrices were also created for each sample year separately, with planktonic respiration being added as a variable in the 2005 matrix. A second matrix, for correlation with the PCA results, consisted of the 24 water quality variables, biodiversity measures (species richness and species evenness) and total phosphorus (TP) (columns) for the lake and pit samples (rows). TP and temperature were included in the second matrix to increase the number of variables that may explain variation in ecosystem function.

The correlation type of PCA was chosen to ensure that all variables are given equal weight. Correlation PCA both centers and standardizes data by unit variance (standard deviation) to produce a cross-product matrix of correlation coefficients where the principal diagonal is made up of ones (self-correlations). The use of correlation PCA is suitable for the phosphorus cycling parameters because phosphate uptake and regeneration tend to show a linear response to the metals that are considered contaminants at high concentrations (e.g. copper, nickel, iron, and zinc) (Kaneko *et al.* 2004).

#### **4.2.3.2 Analysis of Variance (ANOVA) and Multi-Response Permutation Procedures (MRPP)**

Although differences were detected in species composition and abundance between lake groups in Chapter 3, I did not test for differences in biodiversity. Single Factor ANOVA was used to test for significant differences in species richness (a proxy for biodiversity) between the four lake groups. Total planktonic respiration was only available for 2005; therefore a Single Factor ANOVA was run on this data to determine if there were significant differences in planktonic respiration between the 4 lake groups.

MRPP was used to look for significant differences in phosphorus cycling (turnover time of phosphate, particulate phosphorus turnover rate, and planktonic regeneration rate) between the lakes groups determined in Chapter 2. Please see Chapter 2, Section 2.3.2, for an introduction to MRPP.

## 4.3 Results

### 4.3.1 Relationships between Ecosystem Function, Water Quality and Biodiversity

#### Phosphorus Cycling

PCA was performed on a matrix where the columns of the matrix contain three P-cycling variables plus the planktonic respiration variable (2005 sub-matrix only) and the rows contain the study lakes for all sample years. A second matrix of environmental variables (24 water quality variables, species richness, species evenness, TP) (columns) and study lakes for all years (rows) was used to look for indirect relationships between water quality and P-cycling, as well as biodiversity and P-cycling.

Axis 1 of PCA for the combined three years of data is highly representative of the raw data as indicated by the high correlation between ordination distance and raw data distance (Table 4-1). For this reason, only axis 1 is interpreted. The majority of lakes form a cluster around the origin of the ordination signifying that P-cycling properties are similar among these lakes (Fig. 4-1). Only TP is illustrated as a biplot vector when using an  $r^2$  cut-off of 0.250, indicating that none of the 24 water quality variables or biodiversity measures correlate highly with axes 1 and 3. The lakes positioned at the extreme left of the ordination are those that have different P-cycling properties from the majority; they have rapid dissolved P regeneration rates, rapid planktonic turnover rates, and long phosphate turnover times (slow uptake of  $\text{PO}_4$ ) compared to the other lakes in the data set. All three P-cycling measurements were strongly associated with axis 1 (Table 4-1).

**Table 4-1.** Summary statistics for correlation PCA of P-cycling variables based on all three years (analysis of 2005 data including planktonic respiration resulted in identical conclusions, except respiration correlated highest with axis). Correlation between ordination distance and raw-data distance is high for axis 1 making it interpretable (values considered for interpretation are in bold text). Extreme lake scores are lakes positioned at the extreme left of the ordination (Fig. 4-9).

	Axis	Increment Variance (%)	Cumulative Variance (%)
Variance explained in first three axes	<b>1</b>	<b>60.6</b>	<b>60.6</b>
	2	22.2	82.8
	3	17.2	100.0
Correlation between distance and raw data	<b>1</b>	<b>0.672</b>	<b>0.672</b>
	2	-0.037	0.635
	3	0.089	0.724
Highest correlations with P-cycling variables (2003 – 2005)	1	Regeneration Rate, 0.676	
	1	Turnover Time, 0.562	
	1	Turnover Rate, 0.580	
Highest correlations with respiration (2005 only)	1	Planktonic Respiration, 0.515	
Most extreme lake scores	<b>1</b>	<b>B-pit1 -2.2495, B-pit2 -1.3901</b>	
		<b>Sue-C Pit1 -1.3955</b>	
		<b>Upper Link1 -4.7939, Upper Link2 -4.4892</b>	
		<b>Upper Link3 -5.0870</b>	
High correlations with environmental variables*	<b>1</b>	<b>TP, 0.576</b>	

\*R<sup>2</sup> cut-off was set to 0.250

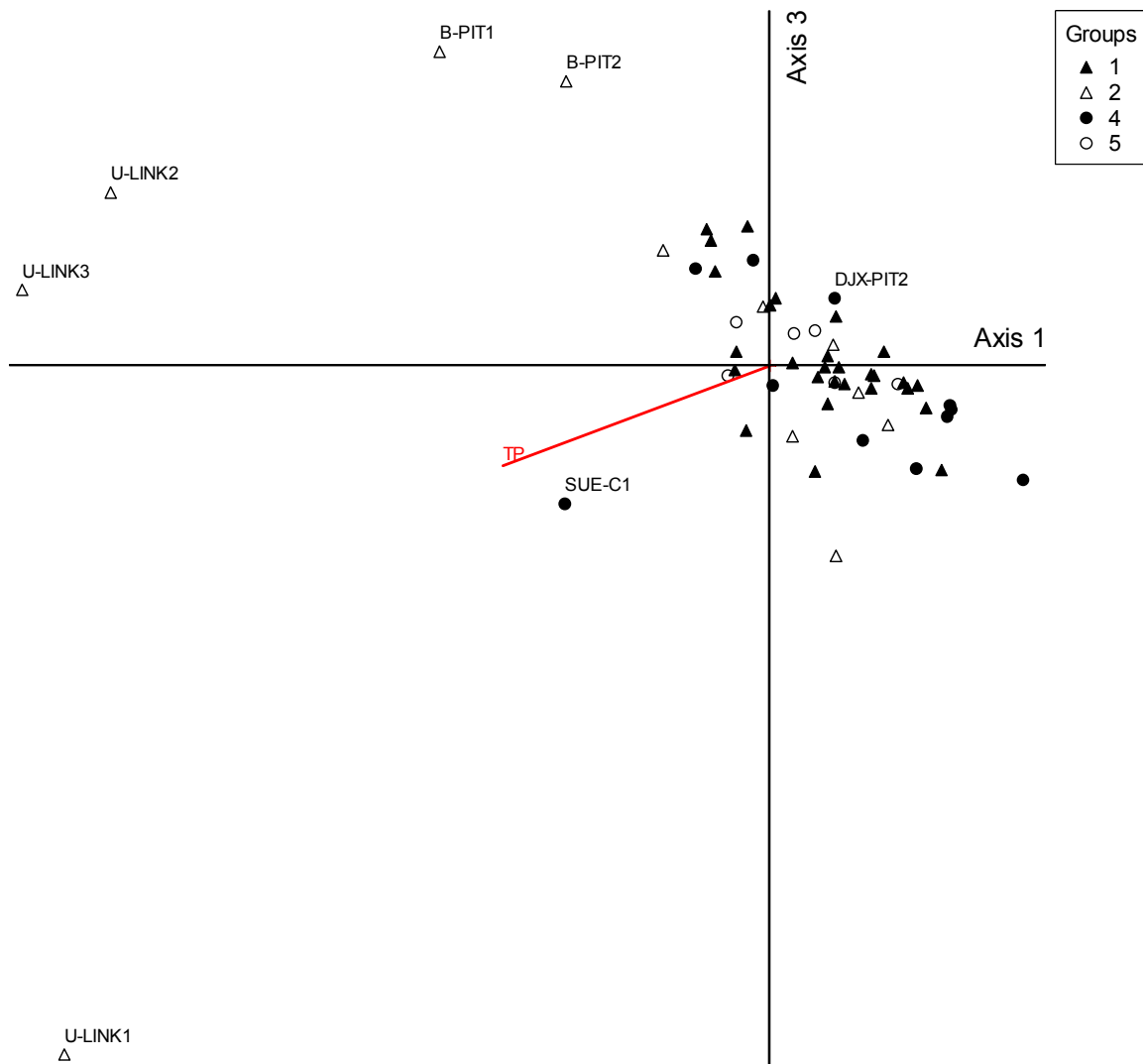
Total phosphorus (TP) is the only variable from the 2<sup>nd</sup> matrix that correlated highly with any axes (0.576, axis 1). Kendall correlations of temperature, species richness and species evenness with axis were much smaller, 0.057, 0.044 and 0.003, respectively. TP was therefore the only variable that was found to correlate with variation in P-cycling properties in the lakes and pits in the data matrix including all years.



Results were similar when PCA was completed for each year separately (results not reported). Axis 1 was consistently the only interpretable axis, while TP was the only variable to correlate highly with axis 1. This was also the case when planktonic respiration was included in the 2005 analysis. Temperature, species richness and species evenness did not show high correlations with any axes in any analyses, even when planktonic respiration was included in the 2005 main matrix. No other water quality variables from the second matrix correlated highly with any ordination axes (Table 4-1) and therefore had no detectable influence on P-cycling for this set of lakes.

Differences in P-cycling between the four lake groups, including samples from all three years, were not detected (MRPP,  $T = -0.166$ ,  $A = 0.004$ ,  $p = 0.374$ ). In addition, respiration rates were not different between lake groups in 2005 (ANOVA,  $p = 0.48$ ). However, differences in species richness were present when comparing all four lake groups (ANOVA,  $p = 0.00003$ , Table 4-2). A pair wise analysis was completed using a Bonferroni test to determine which groups were significantly different from one another (Table 4-2). Mean species richness in groups 2 and 4 were significantly different from the reference group 1 (Table 4-2, Fig. 4-2).

Canonical Correspondence Analysis was run as an independent assessment of the PCA results. CCA searches for the portion of variance in the ecosystem function matrix that can be directly explained by the environmental variables in the second matrix. CCA results support the PCA results as no direct relationships could be found between P-cycling and the 24 water quality variables (Table 4-3).



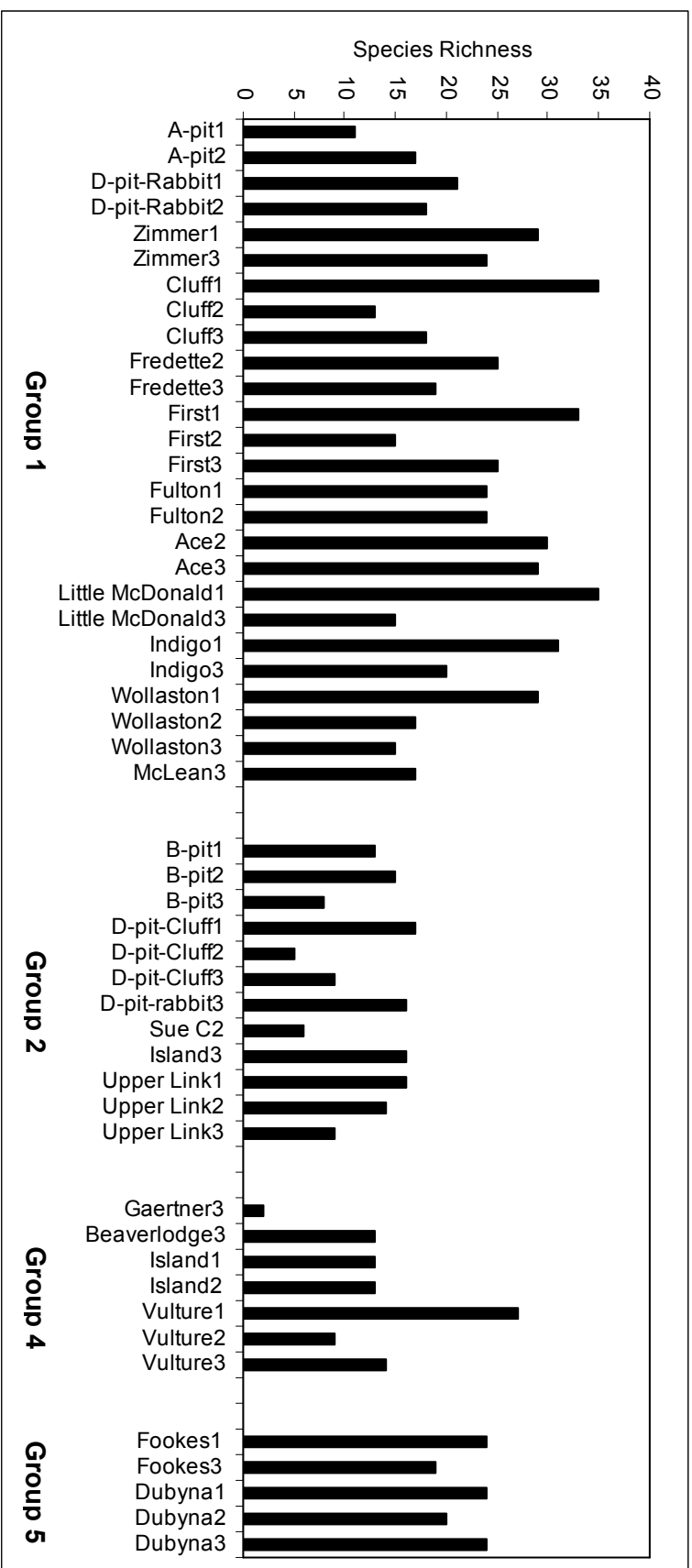
**Figure 4-1.** PCA of P-cycling properties over all years. Axes 1 and 3 are plotted together as they were the most representative of the original data based on correlation between ordination distance and raw data distance (Table 4-1). Six samples are located at the far left of the ordination and show a strong association with axis 1: Upper Link (2003, 2004, and 2005), B-pit (2003, 2004) and Sue-C Pit (2003).

**Table 4-2.** Species richness varies significantly among the four lake groups for the combined data over three years. Subsequent pair wise analysis indicates that lake groups 2 and 4 contain less biodiversity (species richness) than lake group 1. Group 5 species richness is not significantly different from lake group 1.

	Lake Groups	Mean	Variance	p-value
Single Factor ANOVA	1	23.12	45.78	<b>0.00003</b>
	2	11.91	20.49	
	4	14.8	38.57	
	5	21.75	6.92	
Bonferroni Test		<u>p-value</u>		
	1 vs. 2	<b>0.00008</b>		
	1 vs. 4	<b>0.00439</b>		
	1 vs. 5	1.00000		
	2 vs. 4	1.00000		
	2 vs. 5	<b>0.02216</b>		
	4 vs. 5	0.09428		

**Table 4-3.** P-cycling and the water quality parameters were unrelated in this study. The CCA results for all three years below were produced using P-cycling variables to constrain the water quality matrix. None of the axes are representative of the original data, as indicated by the low correlations between ordination and raw data distances.

	Axis	Increment Variance (%)	Cumulative Variance (%)
Variance explained in first three axes	1	58.3	58.3
	2	24.9	83.2
	3	16.8	100.0
Correlation between ordination distance and raw data distance		Increment	Cumulative
	1	0.026	0.026
	2	0.002	0.028
	3	0.000	0.028



**Figure 4-2.** Species richness by lake for the four lake groups over all three years. Lake group 1 contains the lakes and pits that are considered to be reference systems for comparison with other lake groups in this study..

## **4.4 Discussion and Conclusions**

### **4.4.1 Relationships between Ecosystem Function and Water Chemistry**

#### **4.4.1.1 Phosphorus Cycling**

The majority of lakes in the data set exhibit similar planktonic phosphorus cycling and respiration rates. In Chapter 3, water quality was found to influence species composition. In contrast, ecosystem function, as measured in this chapter, appears to be unrelated to water quality. There also were no strong correlations found between species richness, species evenness and water quality. Therefore, biodiversity differences among lake groups also did not appear to be related to water quality.

Only three exposed aquatic systems (Upper Link Lake, B-Pit and Sue-C Pit) had P-cycling rates that were different from the majority of lakes and those differences could not be accounted for by the 24 water quality variables. Rather, total phosphorus seems to account for these differences (Fig. 4-1). This is true for Upper Link Lake and Sue-C pit (2003), which both have high TP compared to all other lakes (Table A5-2 and Table A5-3). However, B-pit TP is comparable to other lakes and pits that had P-cycling rates more typical of the data set. Sue-C pit was removed as an outlier in the 2003 species matrix in Chapter 3 due to its unique species composition and abundance. Therefore, Sue-C pit was not included in the ordination analyse in Chapter 3. However, Sue-C pit (2003) had low species richness (3 identified species) and a strong domination of the 2 – 40 planktonic size fraction. This may be contributing to the observed P-cycling rates, but the TP concentration likely had a stronger influence on these rates than species richness and food web structure.

Mean TP for Upper Link Lake over all three sample years was 1643 nM (range 1491 nM to 1849 nM), and the mean TP for Sue-C Pit over 2003 and 2004 was 1655 nM (range 1398 nM to 1912 nM) (Tables A5-2 and A5-3). These values far exceed the mean TP (397 nM, range 79 – 1912 nM) for all study lakes (Table A5-1 and Table A5-2). These systems also have much higher regeneration rates than the majority of

lakes, which can likely be attributed to the greater TP concentrations, and therefore, greater biomass within the food web. It is generally understood that planktonic regeneration rate increases with TP (Hudson et al. 1999; Nowlin et al. 2007). Hudson et al (1999) illustrated that regeneration is a function of system biomass. However, Upper Link Lake (2003) has a much higher than expected regeneration rate (Table A5-2). But, regardless of the high regeneration rate, the turnover rate of particulate P in Upper Link Lake in 2003 was 40%, which is within the range (10% - 40% day<sup>-1</sup>) of the data set. This suggests that the large regeneration rate in Upper Link may be caused by greater plankton biomass within this lake's food web. This greater planktonic biomass was also accompanied by greater planktonic respiration (143 µg O<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>), compared to the majority of lakes (Table A5-3).

Turnover time of dissolved phosphate was slower in Upper Link Lake in 2004 and 2005, but was comparable in 2003 to the reference and literature lakes. There is seasonal variation in these rate measurements, but within the temporal scale of this study Upper Link Lake does show a tendency to have slow planktonic turnover times. Slow turnover times (2004 and 2005) and rapid regeneration rates (2003) could be an indication of resource use differences within the planktonic community. However, a slow phosphate turnover time may indicate that Upper Link Lake is less P-limited. As lakes become more nutrient rich, there is an expectation that phosphorus will eventually become less limiting (Capblancq 1990; Fisher and Lean 1992; Nowlin et al. 2007) and other nutrients (e.g. nitrogen) may become limiting.

Greater TP concentrations could explain the differences in phosphorus cycling observed in Upper Link and Sue-C pit, but there are also other pits that show slow phosphate turnover times (B-pit, Gaertner Pit, and DJX-Pit, see Table A5-2) that have TP concentrations more typical of the majority of study lakes. These systems are known to be impacted in terms of water quality. However, the 24 water quality variables did not explain the differences seen in phosphorus cycling. Other factors must be influencing the phosphorus cycling in these pits.

Gaertner Pit, B-pit and DJX-pit are strongly dominated by the microbial size fractions and zooplankton is absent from these systems (Fig. 3-17). Such

differences in the size classes may partially explain the differences in phosphorus cycling observed in these pits compared to the majority of the study lakes.

The presence of microbial organisms and low TP should result in rapid nutrient cycling characteristics (Hudson and Taylor 2005; Wen and Shuang-Lin 2003), but this is not the case for Gaertner Pit, B-pit and DJX-pit. Microbial organisms often exhibit greater metabolic rate (Biddanda et al. 2001) and bacteria often out-compete larger plankton for bioavailable nutrients in oligotrophic waters (Del Giorgio et al. 1997). The slow turnover times do not appear to correspond with the size structure of the communities within these three aquatic systems.

The missing size fractions within all three systems may be important for rapid phosphorus uptake. For example, Gaertner Pit and DJX-Pit show a complete absence of organisms larger than 40  $\mu\text{m}$ , whereas B-pit contains all size fractions, except for the >200  $\mu\text{m}$  fraction (Fig. 3-17). The absence of these larger fractions may represent the absence of vital trophic links within these aquatic communities. The missing trophic links could be like missing links in a chain, causing disruptions in nutrient flow pathways. Thus, resource use efficiency may be hindered due to a potential absence of important functional groups within the missing size fractions.

Such explanations are speculative, but the elevated levels of contaminants and unique community size structure do provide explanatory evidence for the atypical P-cycling characteristics within these three aquatic systems. Odum (1985) predicted that chemically stressed ecosystems would possess lower resource use efficiency and that changes in trophic structure would result in hindered bottom-up energy transfer through the food web. Altered trophic structure (i.e. missing size fractions) can act as a bottleneck to energy transfer through an aquatic food web resulting in decreased resource use efficiency (Yan and Strus 1980). In a study exploring multiple indicators of ecosystem health, Xu et al. (1999) describe how stressed ecosystems possess low structural exergy, which is a measure of a systems ability to utilize available resources. As biodiversity decreases with increased chemical stress, food web complexity is reduced resulting in lower resource use efficiency (Xu et al. 1999). Such predictions may explain the P-cycling behaviour observed within B-pit, Gaertner Pit and DJX-pit.

#### **4.4.1.2 Respiration**

Respiration rates were very similar among reference and exposed systems. PCA and CCA results were also in agreement as no direct or indirect relationships could be found between the 24 measured water quality variables and planktonic respiration. Based on the temporal scale of this study and the statistical analyses chosen, mining activities had no detectable effect on planktonic respiration.

The planktonic respiration rates measured in this study were not only similar among lake groups, but were also similar to values reported in the literature. I compared respiration rates from my study to those in three (Arhens and Peters 1991; Carignan et al. 2000; Cammack et al. 2004) other studies that considered temperate dimictic lakes with similar nutrient status (TP) to my study lakes. Despite significant differences in species composition and biodiversity among lake groups, respiration rates are not different among groups and are comparable to values reported in the literature. This supports that mining activities had no detectable effect on planktonic respiration.

#### **4.4.2 Relationships between Ecosystem Function and Biodiversity**

Species richness and evenness did not influence phosphorus cycling or planktonic respiration. Despite significant differences in biodiversity (i.e. richness) among the lakes groups, there was no widespread, detectable effect on planktonic P-cycling or respiration. However, there are potential relationships between planktonic food web structure and ecosystem function within some pits.

The planktonic species richness of Gaertner Pit, B-pit and Sue-C-Pit provide some evidence that low biodiversity and atypical food web structure may be contributing to atypical P-cycling relative to the majority of lakes in this study. The numbers of species present in Sue-C, Gaertner and B-pit are 3, 2, and 12 (mean for all sample years), respectively, whereas, the mean species richness for the reference group is 23. These numbers suggests that there is 87%, 98%, and 48% less richness in Sue-C Pit, Gaertner Pit and B-pit, respectively. There is considerably less biodiversity within these pits compared to the reference systems. Low



biodiversity and missing size fractions from the food web are likely associated with the unusual P-cycling characteristics of these three pits.

Apart from these unique cases, lake exposure to mining activities had no detectable effect on ecosystem function. Furthermore, there were no general relationships found between species composition, species abundance, species richness, and species evenness with ecosystem function. Any differences in function were confined to the three highly impacted aquatic systems, and these differences may result from factors such as nutrient status (TP for this study), seasonal fluctuation, and extremely low biodiversity.

Plankton species composition was significantly different between the four lake groups, as determined in Chapter 3. However, the plankton food webs within each lake group were similar and clustered on the CCA ordinations. This implies that the planktonic food webs developed in response to the type of lake exposure, but ecosystem function was not affected in most lakes and pits. These observations could be explained by functional redundancy theory.

Functional redundancy is often defined as a food web containing multiple species that perform the same function for an ecosystem (Franklin and Mills 2006; Thompson and Starzomski 2007). It is probable that the planktonic food webs in this study, prior to exposure, contained functionally redundant species that were able to tolerate the changing water chemistry within the exposed systems. Even as plankton seasonal succession progresses, a certain amount of functional redundancy must exist. Otherwise, great interseasonal variability would exist in functional measurements, which wasn't the case for the majority of lakes in this study. Other studies suggest that differences in species composition and food-web structure do not necessarily result in functional changes. For example, Franklin and Mills (2006) questioned whether or not changes in microbial community structure and composition would result in measurable functional changes. Through a series of "dilution-induced changes in diversity," bacterial communities were grown with similar abundance, but different community structure and composition. No functional differences could be found among treatments, indicating that functional redundancy was quite prominent.

Functional redundancy is closely tied to the term functional resilience. Nystrom (2006) defined functional resilience as a community's or ecosystem's ability to resist anthropogenic or natural disturbance and remain in the same functional state. Ecosystem function was similar across most lakes and pits in our study regardless of differences in species composition, richness and exposure to mining activities. Thus, according to Nystrom's definition, the majority of lakes in this study are functionally resilient. The composition and diversity within our study systems appear to maintain functional redundancy similar to reference lakes, allowing the planktonic communities to be resilient to environmental stress (differences in water chemistry due to different exposure mechanisms).

The planktonic food webs among exposed lakes either adapted to the various types of mining exposure, or tolerant species simply survived and established the current species composition. However, the few systems that had very low biodiversity exhibited functional characteristics that were different from the majority. Such results support the theories of Lawton and Brown (1994) who implied that species richness is not important until diversity falls well below a certain threshold, at which time the community becomes vulnerable to functional change. Planktonic communities such as that in Gaertner Pit likely do not have the redundancy that is present in more complex and diverse communities. However, the question remains as to what has caused the low diversity in such pits. Is the level of contamination in these pits hindering planktonic community development? If this is the case, then there is an indirect link between exposure and function, as the level of contamination from exposure would explain low species richness, and therefore altered ecosystem function. Or, is low species richness simply a product of recent flooding and recent colonization? This would make it difficult to assess whether or not exposure is important to function. Kalin et al. (2001) found that the phytoplankton community within B-pit (Rabbit Lake Mine Site, same as in our study) experienced rapid changes in species composition and increased richness over a seven year period (1992 – 1998), immediately following flooding. They concluded that these changes were associated with time since flooding and improved water quality (i.e. decreasing trends in As and Ni over the temporal scale of the study). We did not see a similar

increasing trend in richness over the three years that B-Pit was sampled and we did not measure changes in species composition over time. However, the study by Kalin et al. (2001) suggests that it may take time for a complete plankton community to establish in such flooded pits. Regardless, low species diversity in certain pits is associated with ecosystem function characteristics that are different from the majority of lakes and pits in this study.

In conclusion, this study provides evidence that supports the theories of functional redundancy and functional resilience. Planktonic food web structure and biodiversity was similar across most of the exposed aquatic systems and the reference lakes and pits. Accordingly, these lakes and pits also had similar ecosystem function characteristics. These observations were independent of species composition and the mechanism of exposure to mining activities. This study also supports the growing understanding that diversity leads to stability in communities and ecosystems (Downing and Leibold 2002; Duffy et al. 2007; Flombaum and Osvaldo 2008; Gessner et al. 2004; Resetarits and Chalcraft 2007; Thompson and Starzomski 2007). Although the planktonic species composition was different from lake group to lake group, the majority of study systems remained functionally resistant to impact from mining exposure. In contrast, lakes and pits with ecosystem function properties that were different from reference lakes and pits contained planktonic communities with low biodiversity, compared to reference systems.

## **CHAPTER 5. GENERAL CONCLUSIONS**

The aquatic systems sampled during this three year study included lakes and pit-lakes that had been exposed to different types and to different intensities of mining activities. To categorize and manage this type of information, the initial objective of this study was to establish formal lake groups based on water quality. These groups were created using cluster analysis and indicator analysis based on 24 water quality parameters that were measured over all three years for all lake and pit samples. Five separate lake groups were established based on similarities and differences in water quality. Three of these groups were identified based on significant relationships with certain water quality variables associated with exposure to mining activities. The other two groups did not identify with any water quality variables and were merged into a single group. This merged group was categorized as non-impacted, relative to the lakes in other groups, and contained all the reference lakes and some minimally exposed systems. The establishment of these four groups made it possible to look at potential relationships between water quality, species composition, biodiversity and ecosystem function within the planktonic food webs of lakes and flooded pit-lakes exposed to uranium mining activities.

The four lake groups were superimposed onto CCA and NMDS ordinations to assess whether or not they would cluster based on species composition and abundance data for each sampling year. This objective was satisfied, as outlined in Chapter 3. Relationships between species composition and abundance were detected with multivariate ordination analysis. Many of these systems had unique species composition, species richness and food web size structure. Species composition and abundance was associated with the water quality within the lake groups.

The majority of lakes had a food web structure similar to reference lakes. This included lakes and pits that had been exposed to mining activities and are known to have elevated concentrations of some contaminants. The planktonic food webs clearly possess species that are tolerant to the various mechanisms of exposure. Island Lake is likely the best example of community resilience (Loreau 2002).

Although Island Lake's water quality has been heavily impacted from treated effluent, its planktonic food web structure is similar to the reference lakes.

A few lakes and pits had missing size fractions. This may be evidence of impact from elevated concentrations of contaminants, or it may be a product of the young age of the pits which may still be undergoing colonization. It may take time for complex communities to develop because the groundwater and dewatering water used to flood the pits likely carries minimal biota. Plankton species are capable of moving great distances through wind dispersal (Hamilton and Lenton 1998), mechanical transport by boats (Lewis et al. 2003), and waterfowl (Green et al. 2002). Given enough time, these dispersal mechanisms may result in a more diverse and complex set of plankton communities inhabiting these recently flooded pits.

Exposure to mining activities did not have a widespread, detectable effect on ecosystem function for the lakes in this study. In addition, biodiversity measures such as species richness and evenness had no detectable effect on function. A small number of lakes and pits exhibited phosphorus cycling characteristics that were different from the majority of lakes in the data set. However, these characteristics can be explained by factors such as trophic status (total phosphorus), missing size fractions from the food web, strong dominance of certain size fractions and extremely low biodiversity (i.e. species richness).

In conclusion, exposure to various mining activities resulted in significant differences in planktonic species composition and biodiversity among the lake groups. However, such exposure did not result in significant differences in ecosystem function. Only a small number of very unique aquatic systems had ecosystem function properties that were different from the reference group. Among the biodiversity-ecosystem function theories most prevalent in the literature, functional redundancy and resilience best explain the results of this study.

Relationships between biodiversity, ecosystem function and anthropogenic impact are complex when multiple aquatic systems are considered. The conclusions of this study should not be taken as being definitive. However, it considers a number of current issues that are prevalent in a growing debate about biodiversity and ecosystem function in response to anthropogenic disturbance. The current

biodiversity-ecosystem function literature calls for the consideration of more species, more environmental variables, and more ecosystem functions (Hillebrand 2008; Woodward 2008). Further shortcomings in the biodiversity-ecosystem function literature can be identified in studies involving laboratory microcosms experiments that use microbial communities. Such studies use only a fraction of the microbes that are present in natural communities and these experimental communities are manipulated in a laboratory setting (Morin and McGrady-Steed 2004). Attempts to generalise the conclusions of such studies to natural ecosystems would be tenuous. My study addresses the above concerns by considering multiple species of plankton, 24 water quality variables, and two different ecosystem functions. My study also used natural plankton assemblages collected from their natural environment. These considerations make my study novel by providing a realistic and quantitative analysis of the relationships between biodiversity, species composition, and ecosystem function in response to anthropogenic disturbance.

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## APPENDIX 1. Water quality raw data

**Table A1-1.** Water quality data for all lakes that were sampled from 2003-2005, provided by Cameco Corporation and Areva Resources Canada Inc. Lakes denoted with a 1 (i.e. Ace1) contain data from 2003. Lakes denoted with a 2 contain data from 2004, while lakes denoted with a 3 contain data from 2005. There 24 variables measured for each lake. The table has been split into two sections, each of which shows 12 variables across all lakes samples.

Water Quality Variables (12 of 24 variables are shown, concentrations are in mg L <sup>-1</sup> , unless otherwise specified)												
	Temp (°C)	TDS	pH	As <sup>-3</sup> (ug L <sup>-1</sup> )	Al <sup>+3</sup>	Ba <sup>+2</sup>	B <sup>+3</sup>	Ca <sup>+2</sup>	Cl <sup>-</sup>	Cu <sup>+2</sup>	Fe <sup>+2</sup>	HCO <sub>3</sub> <sup>-</sup>
APit1	18.99	64	7.98	1.6	0.008	0.000	0.016	10.00	1.50	0.000	0.042	46
BPit1	19.51	57	7.85	22.0	0.020	0.008	0.023	8.10	0.70	0.000	0.110	34
Cluff1	16.02	98	8.16	0.3	0.005	0.004	0.020	14.00	2.80	0.000	0.066	76
Delta1	20.01	480	7.00	0.0	0.000	0.011	0.000	90.00	30.00	0.000	0.139	65
DPit(C)1	19.85	99	8.38	4.0	0.006	0.004	0.051	13.00	2.10	0.000	1.190	82
DPit(R)1	19.40	94	8.72	5.5	0.000	0.003	0.020	16.00	2.00	0.000	0.058	72
Dubyna1	16.50	119	7.93	0.0	0.000	0.000	0.000	30.00	0.60	0.000	0.013	83
First 1	21.22	115	8.63	0.5	0.000	0.007	0.018	19.00	7.90	0.000	0.120	104
Fookes1	20.13	239	8.87	0.0	0.000	0.025	0.000	22.00	4.00	0.000	0.012	129
Fulton1	18.21	82	8.15	0.0	0.000	0.000	0.000	17.00	0.60	0.000	0.015	71
Indigo1	14.61	11	6.58	0.2	0.012	0.004	0.004	1.70	0.20	0.000	0.510	11
SueC1	20.00	479	4.48	0.0	0.000	0.000	0.000	1.73	0.20	0.000	0.000	8
LiIMac1	12.93	54	7.09	0.0	0.000	0.045	0.000	7.70	10.00	0.066	0.880	29
U- Link1	19.38	42	7.43	3.3	0.025	0.060	0.000	6.40	1.30	0.001	1.270	32
Vulture1	14.09	338	7.85	2.0	0.021	0.026	0.130	89.00	16.00	0.000	0.089	9
Wolly1	15.74	22	7.46	0.3	0.011	0.004	0.005	4.90	0.70	0.000	0.270	7
Zimmer1	19.96	14	7.41	0.0	0.000	0.007	0.000	1.97	0.10	0.000	0.203	13

Table A1-1 continued

	Temp (°C)	TDS	pH	As <sup>-3</sup> (ug L <sup>-1</sup> )	Al <sup>+3</sup>	Ba <sup>+2</sup>	B <sup>+3</sup>	Ca <sup>+2</sup>	Cl <sup>-</sup>	Cu <sup>+2</sup>	Fe <sup>+2</sup>	HCO <sub>3</sub> <sup>-</sup>
APit2	16.00	85	7.56	1.1	0.011	0.004	0.014	10.00	1.70	0.000	0.060	49
Bpit2	16.00	88	7.12	12.0	0.000	0.010	0.025	8.40	0.70	0.000	0.440	35
Dpit(R)2	16.50	88	7.16	5.8	0.007	0.005	0.021	16.00	2.20	0.000	0.220	77
U-Link2	17.90	36	6.86	2.8	0.024	0.001	0.000	6.30	1.00	0.001	1.520	31
Indigo2	12.60	27	6.78	0.2	0.013	0.004	0.000	1.80	0.20	0.000	0.690	12
Vulture2	12.60	606	6.88	2.1	0.000	0.031	0.160	115.00	17.00	0.000	0.065	7
SueC2	11.75	68	7.14	45.0	0.000	0.028	0.000	6.00	4.80	0.017	0.370	26
Dpit(C)2	19.80	105	6.80	5.1	0.008	0.005	0.037	15.00	2.30	0.000	1.000	30
Ace2	18.00	56	7.77	0.1	0.000	0.020	0.000	14.00	0.70	0.000	0.043	49
Dubyna2	20.00	143	7.57	1.6	0.000	0.054	0.000	31.00	0.70	0.000	0.016	89
Fookes2	20.00	240	8.37	1.1	0.000	0.027	0.000	23.00	4.00	0.000	0.019	148
Fulton2	20.00	82	7.92	0.8	0.000	0.000	0.000	18.00	0.70	0.000	0.019	71
Fredet2	17.80	82	7.92	0.6	0.000	0.000	0.000	18.00	0.70	0.000	0.019	71
Wolly2	13.20	22	6.83	0.2	0.000	0.004	0.000	2.90	0.60	0.000	0.069	12
Mclean2	11.90	168	7.13	0.7	0.000	0.009	0.035	29.00	4.10	0.000	0.140	11
Cluff2	20.90	103	8.02	0.2	0.000	0.005	0.021	15.00	3.20	0.000	0.076	78
First2	20.20	114	8.08	0.5	0.005	0.007	0.018	19.00	7.90	0.000	0.120	104
Ace3	16.48	61	7.98	0.0	0.000	0.000	0.000	13.00	0.90	0.000	0.008	50
Bpit3	19.25	88	8.20	11.0	0.026	0.008	0.021	9.00	0.50	0.000	0.120	41
Beaver3	14.77	154	8.09	0.3	0.000	0.000	0.000	21.00	12.00	0.000	0.006	81
Cluff3	16.00	96	8.16	0.0	0.000	0.005	0.022	14.50	3.70	0.000	0.045	79
Delta3	12.91	346	6.29	0.0	0.005	0.011	0.000	73.30	7.00	0.000	0.132	4
Dpit(C)3	16.03	91	7.58	4.5	0.000	0.000	0.000	12.00	2.00	0.000	1.500	76
Dpit(R)3	19.25	88	8.20	2.5	0.028	0.004	0.018	15.00	1.90	0.000	0.100	71
Dubyna3	16.08	143	7.79	0.0	0.000	0.047	0.000	34.00	0.90	0.000	0.015	99
First3	17.14	114	8.13	0.0	0.000	0.007	0.018	19.00	7.90	0.000	0.120	104

**Table A1-1 continued**

	Temp (°C)	TDS	pH	As <sup>-3</sup> (ug L <sup>-1</sup> )	Al <sup>+3</sup>	Ba <sup>+2</sup>	B <sup>+3</sup>	Ca <sup>+2</sup>	Cl <sup>-</sup>	Cu <sup>+2</sup>	Fe <sup>+2</sup>	HCO <sub>3</sub> <sup>-</sup>
Fookes3	16.94	240	8.57	0.0	0.000	0.032	0.000	24.00	4.00	0.000	0.015	159
Fredet3	16.25	56	8.06	0.0	0.000	0.000	0.000	18.00	0.70	0.000	0.019	71
Gaert3	13.78	282	7.33	0.0	0.000	0.000	0.000	62.10	1.90	0.000	0.215	5
Indigo3	14.56	10	7.33	0.0	0.012	0.003	0.000	1.70	0.10	0.000	0.670	10
LilMac3	14.52	32	7.88	0.0	0.000	0.000	0.000	1.03	0.00	0.009	0.092	8
McLean3	14.62	32	7.68	0.0	0.016	0.002	0.025	1.30	0.20	0.000	0.310	7
U- Link3	19.02	36	7.18	1.7	0.031	0.005	0.005	5.60	1.10	0.002	0.890	28
Vulture3	13.66	448	8.70	0.0	0.008	0.082	0.120	98.00	13.00	0.000	0.100	18
Wolly3	9.86	22	7.27	0.1	0.005	0.004	0.002	3.20	0.60	0.000	0.020	13
Zimmer3	14.50	13	7.71	0.0	0.007	0.000	0.000	1.90	0.00	0.000	0.220	11

**Table A1-1 continued (the remaining 12 water quality variables are shown from this point forward)**

	K <sup>+</sup>	Mg <sup>+2</sup>	Mn <sup>+2</sup>	Na <sup>+</sup>	Ni <sup>+2</sup>	Mo <sup>+6</sup>	Ra226 <sup>+2</sup> (Bq L <sup>-1</sup> )	Se <sup>-2</sup>	SO <sub>4</sub> <sup>-2</sup>	U <sup>+3</sup> (ug L <sup>-1</sup> )	Zn <sup>+2</sup>	Hard- ness
APit1	2.10	3.50	0.00	2.90	0.000	0.012	0.0006	0.0001	8.4	38.0	0.000	39
BPit1	1.90	3.40	0.01	2.60	0.150	0.054	0.0300	0.0002	14.0	10.0	0.000	34
Cluff1	0.70	8.80	0.00	2.70	0.003	0.000	0.0050	0.0004	9.7	1.0	0.007	71
Delta1	5.00	7.18	0.15	9.00	0.006	0.085	0.0100	0.0020	240.0	2.0	0.000	90
DPit(C)1	1.30	8.70	0.14	3.40	0.007	0.002	0.0100	0.0001	5.6	47.0	0.000	68
DPit(R)1	2.50	5.50	0.20	3.90	0.000	0.021	0.0060	0.0001	11.0	120.0	0.000	63
Dubyna1	0.50	4.90	0.00	1.90	0.000	0.000	0.0300	0.0001	24.0	350.0	0.000	95
First 1	1.10	11.00	0.03	2.60	0.000	0.000	0.0080	0.0001	1.6	0.0	0.000	93
Fookes1	1.10	4.30	0.00	47.00	0.000	0.000	0.8000	0.0001	52.0	480.0	0.000	73
Fulton1	0.70	4.00	0.00	1.70	0.000	0.000	0.0000	0.0001	4.2	1.8	0.000	59

Table A1-1 continued

	K <sup>+</sup>	Mg <sup>+2</sup>	Mn <sup>+2</sup>	Na <sup>+</sup>	Ni <sup>+2</sup>	Mo <sup>+6</sup>	Ra226 <sup>+2</sup> (Bq L <sup>-1</sup> )	Se <sup>-2</sup>	SO <sub>4</sub> <sup>-2</sup>	U <sup>+3</sup> (ug L <sup>-1</sup> )	Zn <sup>+2</sup>	Hard- ness
Indigo1	0.30	0.60	0.02	1.10	0.000	0.000	0.0000	0.0001	0.6	0.0	0.000	7
SueC1	0.39	0.70	0.01	1.10	0.011	0.000	0.0000	0.0001	2.8	3.0	0.000	7
LilMac1	5.30	3.30	0.00	5.70	0.110	0.200	0.4300	0.0015	9.5	97.0	0.360	31
U- Link1	0.00	3.30	0.04	2.00	0.001	0.008	0.1800	0.0004	3.8	140.0	0.000	30
Vulture1	7.30	6.90	0.24	15.00	0.004	0.710	0.0000	0.0006	258.0	0.2	0.000	250
Wolly1	0.60	0.80	0.02	1.50	0.000	0.015	0.0100	0.0002	12.0	0.2	0.000	16
Zimmer1	0.57	0.95	0.02	1.00	0.000	0.019	0.0000	0.0001	0.7	0.0	0.000	9
APit2	2.00	3.60	0.01	2.80	0.000	0.011	0.0100	0.0001	8.0	31.0	0.010	40
Bpit2	1.90	3.50	0.03	2.50	0.170	0.053	0.0200	0.0002	14.0	9.0	0.000	35
Dpit(R)2	2.50	5.70	0.21	3.80	0.002	0.019	0.0100	0.0001	8.5	110.0	0.011	63
U-Link2	0.90	3.10	0.03	1.30	0.001	0.008	0.1800	0.0003	2.8	112.0	0.000	28
Indigo2	0.30	0.60	0.03	1.20	0.000	0.000	0.0000	0.0001	0.5	0.0	0.000	7
Vulture2	10.00	9.00	0.26	27.00	0.006	0.270	0.0060	0.0006	360.0	0.4	0.000	324
SueC2	2.20	2.20	0.00	4.00	0.057	0.094	0.2000	0.0008	6.0	23.0	0.022	24
Dpit(C)2	1.30	10.00	0.08	3.50	0.007	0.001	0.0300	0.0001	6.2	48.0	0.000	76
Ace2	0.60	2.80	0.00	1.30	0.000	0.000	0.0200	0.0001	6.1	17.0	0.000	46
Dubyna2	0.60	5.30	0.00	2.00	0.000	0.000	0.0400	0.0001	27.0	330.0	0.000	99
Fookes2	1.20	4.60	0.00	50.00	0.000	0.000	1.0000	0.0001	54.0	520.0	0.000	76
Fulton2	0.70	4.10	0.00	1.70	0.000	0.000	0.0000	0.0001	4.1	1.3	0.000	62
Fredet2	0.70	4.10	0.00	1.70	0.000	0.000	0.0000	0.0001	4.1	1.3	0.000	62
Wolly2	0.60	0.90	0.01	1.30	0.000	0.003	0.0000	0.0001	4.1	0.1	0.000	11
Mclean2	2.60	2.60	0.01	7.60	0.000	0.064	0.0060	0.0001	89.0	0.0	0.000	83
Cluff2	0.70	9.40	0.00	2.70	0.003	0.000	0.0060	0.0001	12.0	1.0	0.000	76
First2	1.10	11.00	0.03	2.60	0.000	0.000	0.0080	0.0001	1.6	0.5	0.000	93

Table A1-1 continued

	K <sup>+</sup>	Mg <sup>+2</sup>	Mn <sup>+2</sup>	Na <sup>+</sup>	Ni <sup>+2</sup>	Mo <sup>+6</sup>	Ra226 <sup>+2</sup> (Bq L <sup>-1</sup> )	Se <sup>-2</sup>	SO <sub>4</sub> <sup>-2</sup>	U <sup>+3</sup> (ug L <sup>-1</sup> )	Zn <sup>+2</sup>	Hard- ness
Ace3	0.40	2.60	0.00	1.30	0.000	0.000	0.0300	0.0001	5.7	12.0	0.000	43
Bpit3	1.70	3.60	0.04	2.60	0.120	0.050	0.0600	0.0001	14.0	11.0	0.000	37
Beaver3	1.10	5.10	0.00	20.00	0.002	0.000	0.0400	0.0023	33.0	160.0	0.000	73
Cluff3	0.70	9.20	0.00	2.75	0.002	0.000	0.0050	0.0001	10.5	1.0	0.000	74
Delta3	5.93	6.56	0.07	8.33	0.000	0.097	0.0200	0.0001	210.0	0.0	0.000	210
Dpit(C)3	1.20	8.00	0.30	2.90	0.007	0.002	0.0100	0.0001	6.6	59.0	0.000	63
Dpit(R)3	2.10	5.30	0.07	3.40	0.003	0.019	0.0100	0.0001	11.0	95.0	0.000	59
Dubyna3	0.70	5.90	0.00	2.30	0.000	0.000	0.0700	0.0001	30.0	330.0	0.000	109
First3	1.10	11.00	0.03	2.60	0.000	0.000	0.0080	0.0001	1.6	0.5	0.000	93
Fookes3	1.20	4.70	0.00	51.00	0.000	0.000	1.0000	0.0001	54.0	560.0	0.000	79
Fredet3	0.70	4.10	0.00	1.70	0.000	0.000	0.0000	0.0001	4.1	1.3	0.000	62
Gaert3	1.00	7.12	0.52	3.00	1.620	0.000	0.3300	0.0020	163.0	219.0	0.155	89
Indigo3	0.20	0.60	0.02	1.00	0.000	0.000	0.0000	0.0001	0.5	0.0	0.000	7
LilMac3	1.00	0.40	0.02	1.78	0.009	0.000	0.0000	0.0001	2.9	0.0	0.007	4
McLean3	0.30	0.40	0.03	1.00	0.000	0.043	0.0000	0.0001	0.5	0.0	0.000	5
U- Link3	0.60	2.80	0.03	1.50	0.001	0.010	0.2000	0.0002	3.7	130.0	0.000	25
Vulture3	8.10	6.60	0.21	21.00	0.011	0.120	0.0100	0.0006	280.0	13.0	0.000	272
Wolly3	0.60	1.10	0.01	1.40	0.000	0.000	0.0000	0.0001	4.2	0.4	0.000	13
Zimmer3	1.21	0.89	0.02	1.43	0.000	0.000	0.0300	0.0001	0.8	0.0	0.000	8

## APPENDIX 2. Lake Groups and Associated Indicator Variables

**Table A2-1.** Concentrations of indicator variables for group 1. Lake group 1 is compared to group 3, as per the separate analysis used to determine why these two groups were clustered into two separate groups. Concentrations are often several times higher within group 1 lakes and pits than in group 3, supporting that indicator analysis was successful in identifying the appropriate variables for each group. All concentration units are  $\text{mg L}^{-1}$ , unless otherwise stated.

GROUP 1 LAKES	Temp	pH	Ca <sup>+2</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Mg <sup>+2</sup>	U <sup>+3</sup> ( $\mu\text{g L}^{-1}$ )	Hardness
Ace2	18.00	7.77	14.00	0.7	49	2.8	17.00	46
Ace3	16.48	7.98	13.00	0.9	50	2.6	12.00	43
APit1	18.99	7.98	10.00	1.5	46	3.5	38.00	39
APit2	16.00	7.56	10.00	1.7	49	3.6	31.00	40
Cluff1	16.02	8.16	14.00	2.8	76	8.8	1.00	71
Cluff2	20.90	8.02	15.00	3.2	78	9.4	1.00	76
Cluff3	16.00	8.16	14.50	3.7	79	9.2	0.95	74
DPit(R)1	19.40	8.72	16.00	2.0	72	5.5	120.00	63
Dpit(R)2	16.50	7.16	16.00	2.2	77	5.7	110.00	63
First 1	21.22	8.63	19.00	7.9	104	11.0	0.00	93
First2	20.20	8.08	19.00	7.9	104	11.0	0.50	93
First3	17.14	8.13	19.00	7.9	104	11.0	0.50	93
Fredet2	17.80	7.92	18.00	0.7	71	4.1	1.30	62
Fredet3	16.25	8.06	18.00	0.7	71	4.1	1.30	62
Fulton1	18.21	8.15	17.00	0.6	71	4.0	1.80	59
Fulton2	20.00	7.92	18.00	0.7	71	4.1	1.30	62
LilMac1	20.00	7.00	1.73	0.2	8	0.7	3.00	7
Zimmer1	19.96	7.41	1.97	0.1	13	0.9	0.00	9
GROUP 1 Avg.	18.28	7.93	14.12	2.5	66	5.7	18.93	59
GROUP 3 Avg.	13.61	7.26	6.71	0.9	10	1.0	0.07	21
<b>GROUP 1 Avg./GROUP 3 Avg.</b>	<b>1.34</b>	<b>1.09</b>	<b>2.10</b>	<b>2.8</b>	<b>6</b>	<b>5.6</b>	<b>270.36</b>	<b>3</b>

**Table A2-2.** Concentrations of significant (p-values  $\leq 0.05$ ) indicator variables for group 2. These values are divided by the means of all other lakes not included in group 2. The resulting values (GROUP avg./ALL OTHER LAKES avg.) represent how many times greater the concentrations of the indicator variables are within the group 2 lakes and pits compared to all other lakes. All concentration units are  $\text{mg L}^{-1}$ , unless otherwise stated.

<b>GROUP 2 LAKES</b>	<b>As<sup>-3</sup> (<math>\mu\text{g L}^{-1}</math>)</b>	<b>Al<sup>+3</sup></b>	<b>Fe<sup>+2</sup></b>
BPit1	22.0	0.020	0.11
Bpit2	12.0	0.000	0.44
Bpit3	11.0	0.026	0.12
DPit(C)1	4.0	0.006	1.19
Dpit(C)2	5.1	0.008	1.00
Dpit(C)3	4.5	0.000	1.50
Dpit(R)3	2.5	0.028	0.10
SueC2	45.0	0.000	0.37
Island3	0.2	0.000	1.60
U- Link1	3.3	0.025	1.27
U-Link2	2.8	0.024	1.52
U- Link3	1.7	0.031	0.89
GROUP 2 avg.	9.5	0.014	0.84
ALL OTHER LAKES avg.	0.8	0.004	0.14
<b>GROUP 4 avg./ALL OTHER LAKES avg.</b>	<b>11.9</b>	<b>3.750</b>	<b>6.18</b>

**Table A2-3.** Concentrations of indicator variables for group 3. Lake group 3 is compared to group 1, as per the separate analysis used to determine why these two groups were clustered into two separate groups. Concentrations are often several times higher within group 3 lakes than in group 1, supporting that indicator analysis was successful in identifying the appropriate variables for each group. All concentration units are  $\text{mg L}^{-1}$ .

<b>GROUP 3 LAKES</b>	<b>Al<sup>+3</sup></b>	<b>Fe<sup>+2</sup></b>
Indigo1	0.012	0.510
Indigo2	0.013	0.690
Indigo3	0.012	0.670
LilMac3	0.000	0.092
McLean2	0.000	0.140
McLean3	0.000	0.170
Wolly1	0.011	0.270
Wolly2	0.000	0.069
Wolly3	0.005	0.020
Zimmer3	0.007	0.220
GROUP 3 Avg.	0.006	0.285
GROUP 1 Avg.	0.002	0.070
<b>Group 3 Avg./Group 1 Avg.</b>	<b>3.000</b>	<b>4.097</b>

**Table A2-4.** Concentrations of significant (p-values  $\leq 0.05$ ) indicator variables for group 4. These values are divided by the means of all other lakes not included in group 4. The resulting values (GROUP avg./ALL OTHER LAKES avg.) represent how many times greater the concentrations of the indicator variables are within the group 4 lakes and pits compared to all other lakes. All concentration units are mg L<sup>-1</sup>.

GROUP 4 LAKES	TDS	B <sup>+3</sup>	Ca <sup>+2</sup>	Cl <sup>-</sup>	K <sup>+</sup>	Mg <sup>+2</sup>	Mn <sup>+2</sup>	Na <sup>+</sup>	Mo <sup>+6</sup>	Se <sup>-2</sup>	SO <sub>4</sub> <sup>-2</sup>	Zn <sup>+2</sup>	Hardness
Delta1	480	0.00	90.0	30.0	5.00	7.18	0.150	9.00	0.085	0.0020	240.0	0.000	90
Delta3	346	0.00	73.3	7.0	5.93	6.56	0.067	8.33	0.097	0.0001	210.0	0.000	210
SueC1	54	0.00	7.7	10.0	5.30	3.30	0.000	5.70	0.200	0.0015	9.5	0.360	31
DJX2	1282	1.10	7.0	2.2	1.00	2.30	1.750	1.40	0.500	0.0030	6.0	0.137	26
Gaert3	282	0.00	62.1	1.9	1.00	7.12	0.517	3.00	0.000	0.0020	163.0	0.155	89
Beaver3	154	0.00	21.0	12.0	1.10	5.10	0.000	20.00	0.000	0.0023	33.0	0.000	73
Island1	2562	0.35	262.0	728.0	15.00	72.00	0.020	515.00	0.660	0.0028	1010.0	0.000	949
Island2	2155	0.30	203.0	525.0	11.00	61.00	0.030	387.00	0.500	0.0020	790.0	0.005	755
Vulture1	338	0.13	89.0	16.0	7.30	6.90	0.240	15.00	0.710	0.0006	258.0	0.000	250
Vulture2	606	0.16	115.0	17.0	10.00	9.00	0.260	27.00	0.270	0.0006	360.0	0.000	324
Vulture3	448	0.12	98.0	13.0	8.10	6.60	0.210	21.00	0.120	0.0006	280.0	0.000	272
GROUP 4 avg.	792	0.20	93.5	123.8	6.43	17.01	0.295	92.04	0.286	0.0016	305.4	0.060	279
ALL OTHER LAKES avg.	119	0.01	13.8	3.6	1.10	4.65	0.033	6.54	0.020	0.0001	16.1	0.001	54
GROUP 4 avg./ALL OTHER LAKES avg.	7	17.41	6.8	34.3	5.85	3.66	8.862	14.06	14.471	11.259	18.9	44.314	5



**Table A2-5** Concentrations of significant (p-values  $\leq 0.05$ ) indicator variables for group 5. These values are divided by the means of all other lakes not included in group 5. The resulting values (GROUP avg./ALL OTHER LAKES avg.) represent how many times greater the concentrations of the indicator variables are within the group 5 lakes and pits compared to all other lakes.

<b>GROUP 5 LAKES</b>	<b>HCO<sub>3</sub><sup>-</sup></b>	<b>U<sup>+3</sup></b>	<b>Ra-226<sup>+2</sup> (Bq L<sup>-1</sup>)</b>	<b>Ba<sup>+2</sup></b>	<b>pH</b>
Fookes1	129	480	0.800	0.025	8.870
Fookes2	148	520	1.000	0.027	8.370
Fookes3	159	560	1.000	0.032	8.570
Dubyna1	83	350	0.030	0.056	7.930
Dubyna2	89	330	0.040	0.054	7.570
Dubyna3	99	330	0.070	0.047	7.790
GROUP 5 avg.	118	428	0.490	0.040	8.183
ALL OTHER LAKES avg.	43	70	0.048	0.011	7.555
<b>GROUP 5 avg./ALL OTHER LAKES avg.</b>	<b>3</b>	<b>6</b>	<b>10.119</b>	<b>3.678</b>	<b>1.083</b>

**Table A2-6** Comparison of individual water quality variables for all four lake groups. Water quality data are summarized using descriptive statistics.

<b>Water Quality Variable</b>	<b>Lake Group</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Standard Deviation</b>
Temp (°C)	1	16.36	9.86	21.22	2.89
	2	17.82	11.75	19.85	2.44
	4	14.53	9.10	21.40	3.42
	5	18.27	16.08	20.13	1.96
TDS (mg L <sup>-1</sup> )	1	65	10	168	41
	2	174	36	1294	353
	4	792	54	2562	842
	5	187	119	240	58

Table A2-6 continued

Water Quality Variable	Lake Group	Mean	Minimum	Maximum	Standard Deviation
pH	1	7.70	6.58	8.72	0.54
	2	7.56	6.80	8.38	0.55
	4	7.42	4.49	9.20	1.30
	5	8.18	7.57	8.87	0.50
As <sup>-3</sup> (ug L <sup>-1</sup> )	1	0.7	0.0	5.8	1.5
	2	9.5	0.2	45.0	12.7
	4	1.3	0.0	3.7	1.5
	5	0.5	0.0	1.6	0.7
Al <sup>+3</sup> (mg L <sup>-1</sup> )	1	0.004	0.000	0.016	0.005
	2	0.014	0.000	0.031	0.013
	4	0.007	0.000	0.033	0.011
	5	0.000	0.000	0.000	0.000
Ba <sup>+2</sup> (mg L <sup>-1</sup> )	1	0.005	0.000	0.045	0.009
	2	0.013	0.000	0.060	0.017
	4	0.026	0.000	0.082	0.023
	5	0.040	0.025	0.056	0.014
B <sup>+3</sup> (mg L <sup>-1</sup> )	1	0.009	0.000	0.035	0.011
	2	0.022	0.000	0.080	0.025
	4	0.196	0.000	1.100	0.325
	5	0.000	0.000	0.000	0.000
Ca <sup>+2</sup> (mg L <sup>-1</sup> )	1	11.1	1.0	29.0	7.7
	2	12.5	5.6	45.0	10.8
	4	93.5	7.0	262.0	79.0
	5	27.3	22.0	34.0	5.0
Cl <sup>-</sup> (mg L <sup>-1</sup> )	1	2.2	0.0	10.0	2.8
	2	8.1	0.5	79.0	22.4
	4	123.8	1.9	728.0	252.8
	5	2.4	0.6	4.0	1.8

**Table A2-6 continued**

<b>Water Quality Variable</b>	<b>Lake Group</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Standard Deviation</b>
Cu <sup>+2</sup> (mg L <sup>-1</sup> )	1	0.003	0.000	0.066	0.013
	2	0.002	0.000	0.017	0.005
	4	0.007	0.000	0.066	0.020
	5	0.000	0.000	0.000	0.000
Fe <sup>+2</sup> (mg L <sup>-1</sup> )	1	0.183	0.008	0.880	0.230
	2	0.843	0.100	1.600	0.587
	4	0.177	0.006	0.880	0.242
	5	0.015	0.012	0.019	0.002
HCO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	1	47	7	104	34
	2	47	26	82	22
	4	30	4	81	26
	5	118	83	159	32
K <sup>+</sup> (mg L <sup>-1</sup> )	1	1.1	0.2	5.3	1.1
	2	1.4	0.0	2.2	0.7
	4	6.4	1.0	15.0	4.5
	5	0.9	0.5	1.2	0.3
Mg <sup>+2</sup> (mg L <sup>-1</sup> )	1	4.1	0.4	11.0	3.6
	2	6.0	2.2	18.0	4.6
	4	17.0	2.3	72.0	24.7
	5	5.0	4.3	5.9	0.6
Mn <sup>+2</sup> (mg L <sup>-1</sup> )	1	0.0256	0.0000	0.2100	0.0518
	2	0.0668	0.0000	0.3000	0.0823
	4	0.2949	0.0000	1.7500	0.5070
	5	0.0010	0.0000	0.0030	0.0015
Na <sup>+</sup> (mg L <sup>-1</sup> )	1	2.3	1.0	7.6	1.5
	2	6.9	1.3	53.0	14.5
	4	92.0	1.4	515.0	179.9
	5	25.7	1.9	51.0	25.9

Table A2-6 continued

Water Quality Variable	Lake Group	Mean	Minimum	Maximum	Standard Deviation
Ni <sup>+2</sup> (mg L <sup>-1</sup> )	1	0.005	0.000	0.110	0.021
	2	0.044	0.000	0.170	0.065
	4	0.246	0.000	1.620	0.534
	5	0.000	0.000	0.000	0.000
Mo <sup>+6</sup> (mg L <sup>-1</sup> )	1	0.015	0.000	0.200	0.039
	2	0.058	0.001	0.400	0.111
	4	0.286	0.000	0.710	0.262
	5	0.000	0.000	0.000	0.000
Ra226 <sup>+2</sup> (Bq L <sup>-1</sup> )	1	0.0212	0.0000	0.4300	0.0806
	2	0.0783	0.0100	0.2000	0.0839
	4	0.1243	0.0000	0.5030	0.1948
	5	0.4900	0.0300	1.0000	0.4913
Se <sup>-2</sup> (mg L <sup>-1</sup> )	1	0.0002	0.0001	0.0015	0.0003
	2	0.0002	0.0001	0.0008	0.0002
	4	0.0016	0.0001	0.0030	0.0010
	5	0.0001	0.0001	0.0001	0.0000
SO <sub>4</sub> <sup>-2</sup> (mg L <sup>-1</sup> )	1	8.1	0.5	89.0	16.3
	2	19.0	2.8	140.0	38.3
	4	305.4	6.0	1010.0	319.7
	5	40.2	24.0	54.0	14.6
U <sup>+3</sup> (ug L <sup>-1</sup> )	1	15.5	0.0	120.0	34.4
	2	57.4	5.0	140.0	49.9
	4	232.1	0.0	1800.0	526.1
	5	428.3	330.0	560.0	103.8

**Table A2-6 continued**

<b>Water Quality Variable</b>	<b>Lake Group</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Standard Deviation</b>
Zn <sup>+2</sup> (mg L <sup>-1</sup> )	1	0.014	0.000	0.360	0.068
	2	0.002	0.000	0.022	0.006
	4	0.060	0.000	0.360	0.115
	5	0.000	0.000	0.000	0.000
Hardness (mg L <sup>-1</sup> )	1	44	4	93	31
	2	55	24	186	45
	4	279	26	949	304
	5	89	73	109	15

### APPENDIX 3. Species Keys and Species Composition/Abundance Matrices

**Table A.3-1.** The following tables contain all species found among all lakes for each sampling year. The full names provided by the identification company are provided along with a key of abbreviated names that were used in the multivariate analyses. These keys had to be constructed due to text limitations in the PC-ORD software used to perform the analyses. Not all of the species found in these tables are present in the data matrices used for the analyses because rare species and outlying species were deleted prior to analysis to improve normality.

#### 2003 Plankton Species Key For Multivariate Analysis

Identification	Key	Identification	Key
<i>Achnanthes</i> spp.	ACHNAN	<i>Keratella</i>	KERATELA
<i>Anabaena spiroides</i>	ANABAE-S	<i>Lyngbya</i> spp.	LYNGBYA
<i>Anabaena</i> spp.	ANABAE	<i>Mallomonas caudata</i>	MALLOMO
<i>Ankistrodesmu falcatus</i>	ANKIST-F	<i>Merismopedia elegans</i>	MERIS-M
<i>Aphanocapsa</i> spp.	APHANO	<i>Monoraphidium</i> spp.	MONORA
<i>Aphanothece nidulans</i>	APHATH-N	<i>Mougeotia</i> sp. (small)	MOUGEO-S
<i>Asterionella formosa</i>	ASTERIO	Nauplius larvae	NAUPLIUS
<i>Arthrodesmus incus</i>	ATHROD-I	<i>Navicula</i> spp.	NAVICULA
<i>Bitrichia chodatii</i>	BRITRICH	<i>Nitzschia</i> spp. (small)	NITZ-S
<i>Botryococcus braunii</i>	BOTRYOC	<i>Oocystis lacustris</i>	OOCYST-L

**Table A3-1 continued (2003)**

Identification	Key	Identification	Key
<i>Ceratium hirundinella</i>	CERATIUM	<i>Peridinium inconspicuum</i>	PERIDI-I
<i>Chlamydomonas</i> spp.	CHLAMID	<i>Peridinium</i> spp.	PERDI
<i>Chroococcus minutus</i>	CHROOC-M	<i>Polyarthra</i>	POLYART
<i>Chroococcus</i> spp.	CHROOC	<i>Pseudanabaena</i> spp. (small)	PSEUD-S
<i>Chrysolykos planctonicus</i>	CHRYSOLEY	<i>Quadrigula lacustris</i>	QUADRIG
<i>Chrysosphaerella longispina</i>	CHRYSOSP	<i>Rhabdoderma lineare</i>	RHABDO-L
Ciliate sp. 1	CILIATE1	<i>Rhizosolenia eriensis</i>	RHIZOS-E
Ciliate sp. 2	CILIATE2	<i>Rhodomonas lacustris</i>	RHODO-L
<i>Cryptomonas erosa</i>	CRYPTO-E	<i>Rhodomonas nannoplanktonica</i>	RHODO-N
<i>Cryptomonas marssonii</i>	CRYPTO-M	<i>Rhopalodia</i> spp.	RHOPA
<i>Cryptomonas rostriformis</i>	CRYPTO-R	<i>Rotifer</i>	ROTIFERS
<i>Cyclotella kutziana</i>	CYCLOT-K	<i>Roya obtusum</i>	ROYA

**Table A3-1 continued (2003)**

Identification	Key	Identification	Key
<i>Cyclotella meneghiniana</i>	CYCLOT-M	<i>Spondylosium planum</i>	SPONDYL
<i>Cyclotella</i> spp. (large)	CYCLOT	<i>Synedra</i> spp. (large)	SYNED-L
<i>Daphnia</i>	DAPHNIA	<i>Synedra</i> spp. (medium)	SYNED-M
<i>Dinobryon bavaricum</i>	DINO-B	<i>Synedra ulna</i>	SYNED-U
<i>Dinobryon divergens</i>	DINO-D	<i>Tabellaria fenestrata</i>	TABEL-FE
<i>Epipyxis</i> spp.	EPIPYXIS	<i>Tabellaria flocculosa</i>	TABEL-FL
<i>Eudorina</i> sp.	EUDOR	<i>Tetraedron minimum</i>	TETRAED
<i>Euglena</i> spp.	EUGLEN		
<i>Geitlerinema</i> spp.	GEITLERI		
<i>Glenodinium</i> spp.	GLENOD		
<i>Gloeocystis</i> sp.	GLEOCYST		
<i>Gymnodinium</i> sp. 1	GYMNOD1		
<i>Gymnodinium</i> sp. 2	GYNMOD2		
<i>Gyromitus cordiformis</i>	GYROMI-C		
<i>Katablepharis ovalis</i>	KATABL-O		



**Table A3-1 continued (2003)**

Identification	Key	Identification	Key
<i>Katablepharis</i> spp.	KATABL		
<i>Kellicottia</i> sp.	KELLICOT		
<i>Kephyrion</i> spp.	KEPHYR		

**2004 Plankton Species Key For Multivariate Analysis**

Identification	Key	Identification	Key
<i>Achnanthes minutissima</i>	ACHNAN	<i>Katablepharis</i> spp.	KATABL
<i>Amoebae</i>	AMOEBAE	<i>Kellicottia</i>	KELLICOT
<i>Anabaena spiroides</i>	ANABAE-S	<i>Kephyrion</i> spp.	KEPHYR
<i>Anabaena spiroides</i> (cells)	ANABAE-C	<i>Keratella</i>	KERATELA
<i>Aphanizomenon flos-aquae</i>	APHANI-F	<i>Kirschneriella</i> spp.	KIRSCH
<i>Aphanocapsa</i> spp.	APHANO	<i>Limnocalanus</i>	LIMNOCA
<i>Aphanothece nidulans</i>	APHATH-N	<i>Lyngbya</i> spp.	LYNGBYA
<i>Aphanothece</i> spp.	APHATH	<i>Mallomonas caudata</i>	MALLOMO
<i>Arthrodesmus incus</i>	ATHROD-I	<i>Mallomonas</i> spp.	MALLOMOS
<i>Asterionella formosa</i>	ASTERIO	<i>Melosira</i> spp.	MELOS

**Table A3-1 continued (2004)**

Identification	Key	Identification	Key
<i>Bitrichia chodatii</i>	BRITRICH	<i>Melosira</i> spp. (large)	MELOS-L
<i>Bosmina</i>	BOSMINA	<i>Merismopedia glauca</i>	MERIS-G
<i>Botryococcus braunii</i>	BOTRYOC	<i>Merismopedia minutissima</i>	MERIS-M
<i>Ceratium hirundinella</i>	CERATUM	<i>Microcystis aeruginosa</i>	MICROCYC
<i>Chlamydomonas</i> spp.	CHLAMID	<i>Monoraphidium</i> spp.	MONORA
<i>Chlamysomonas</i> / <i>Carteria</i> spp.	CHLAMID-C	<i>Nauplii larvae</i>	NAUPLIUS
<i>Chromulina</i> spp.	CHROMUL	<i>Navicula</i> spp.	NAVICULA
<i>Chroococcus dispersus</i>	CHROOC-M	<i>Nematodes</i>	NEMATODE
<i>Chroomonas minutus</i>	CHROOC-D	<i>Nephrocytium lunatum</i>	NEPHRO
<i>Chrysochromulina parva</i>	CHRYSOCH	<i>Nitzschia gracilis</i>	NITZ-G
<i>Chrysolykos planctonica</i>	CHRYSOLY	<i>Nitzschia</i> spp. (small)	NITZ-S
<i>Ciliate sp. 1</i>	CILIATE1	<i>Ochromonas</i> spp.	OCHROM
<i>Ciliate sp. (large - stalked)</i>	CILIATE-L	<i>Oedogonium</i> sp. (small sp.)	OEDOGO

**Table A3-1 continued (2004)**

Identification	Key	Identification	Key
<i>Closterium lunatum</i>	CLOSTER	<i>Oocystis lacustris</i>	OOCYST-L
<i>Coelastrum microporum</i>	COELAST	<i>Pediastrum boryanum</i>	PEDIAS
<i>Coelosphaerium kuetzingianum</i>	COELOS-K	<i>Peridinium cinctum</i>	PERIDI-C
<i>Coelosphaerium</i> spp. (large)	COELOS-L	<i>Peridinium</i> sp.	PERIDI
<i>Coelosphaerium</i> spp. (small)	COELOS-S	<i>Polyarthra</i>	POLYART
Colonial Bluegreen sp.	COLONY	<i>Pseudanabaena</i> spp.	PSEUD-S
<i>Cosmarium</i> spp.	COSMARI	<i>Pseudokephyrion</i> spp.	PSEUDO
<i>Crucigenia</i> spp. (cf. <i>C. quadrata</i> )	CRUCIG	<i>Quadrigula lacustris</i>	QUADRIG
<i>Cryptomonas erosa</i>	CRYPTO-E	<i>Rhabdoderma lineare</i>	RHABDO-L
<i>Cryptomonas platyuris</i>	CRYPTO-P	<i>Rhizosolenia eriensis</i>	RHIZOS-E
<i>Cryptomonas rostriformis</i>	CRYPTO-R	<i>Rhodomonas lacustris</i>	RHODO-L
<i>Cryptomonas</i> spp.	CRYPTO	<i>Rhodomonas minutus</i>	RHODO-M

**Table A3-1 continued (2004)**

Identification	Key	Identification	Key
<i>Cryptomonas</i> spp. (small)	CRYPTO-S	<i>Rhodomonas</i> <i>nannoplanktonicus</i>	RHODO-N
<i>Cyclotella bodanica</i>	CYCLOT-B	<i>Rhopalodia gibba</i>	RHOPA-G
<i>Cyclotella comta</i>	CYCLOT-C	<i>Rhopalodia</i> spp.	RHOPA
<i>Cyclotella</i> <i>meneghiniana</i>	CYCOT-M	<i>Rotifers</i>	ROTIFERS
<i>Cyclotella</i> spp.	CYCLOT	<i>Roya obtusa</i>	ROYA
<i>Diatoma</i> spp.	DIATOMA	<i>Scenedesmus arcuatus</i>	SCENE-A
<i>Dictyosphaerium</i> <i>pulchellum</i>	DICTYO	<i>Scenedesmus bijuga</i>	SCENE-B
<i>Dinobryon</i> <i>bavaricum</i>	DINO-BA	<i>Schroederia setigera</i>	SCHROE-S
<i>Dinobryon borgei</i>	DINO-BO	<i>Schroederia</i> spp.	SCHROE
<i>Dinobryon divergens</i>	DINO-D	<i>Scourfeldia</i> sp.	SCOUR
<i>Dinobryon sociale</i>	DINO-S	<i>Spondylosium planum</i>	SPONDYL
<i>Dinobryon</i> spp. (monods)	DINO	<i>Staurastrum</i> <i>paradoxum</i>	STAURA-P
<i>Dinobryon</i> <i>vanhoffenii</i>	DINO-V	<i>Staurastrum</i> spp.	STAURA
<i>Eubbranchipus</i>	EUBRANC	<i>Stauroneis</i> spp.	STAURO

**Table A3-1 continued (2004)**

<b>Identification</b>	<b>Key</b>	<b>Identification</b>	<b>Key</b>
<i>Euglena</i> spp.	EUGLEN	<i>Synedra</i> spp. (large)	SYNED-L
<i>Fragilaria crotonensis</i>	FRAGIL-C	<i>Synedra</i> spp. (medium)	SYNED-M
<i>Fragilaria</i> spp.	FRAGIL	<i>Tabellaria flocculosa</i>	TABEL-FL
<i>Glenodinium</i> spp.	GLENOD	<i>Temnogametum</i> spp.	TEMNOGA
<i>Glenodinium</i> spp. (small)	GLENOD-S	<i>Tetraedron minimum</i>	TETRAE-M
<i>Gloeocystis</i> sp.	GLEOCYST	<i>Tetraedron trigonum</i>	TETRAE-T
<i>Gomphosphaeria</i> spp.	GOMPHO	<i>Trachelomonas</i> sp.	TRACHEL
<i>Gomphosphaeria</i> spp. (small)	GOMPHO-S		
<i>Gonatozygon</i> sp.	GONATOZ		
<i>Gymnodinium</i> spp.	GYMNOD		
<i>Gymnozygon moniliformis</i>	GYMNOZ		
<i>Gyromitus cordiformis</i>	GYROMI-C		

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**2005 Phytoplankton Species Key For Multivariate Analysis**

Identification	Key	Identification	Key
<b>CYANOBACTERIA</b>		<b>DIATOMS</b>	
<i>Anabaena circinalis</i> Rabenhorst	ANABAE-C	<i>Achnanthes lanceolata</i> (Brebisson) Grunow	ACHNAN-L
<i>Anabaena flos-aquae</i> Brebisson	ANABAE-F	<i>Achnanthes minutissima</i> Kuetzing	ACHNAN-M
<i>Anabaena</i> sp	ANABAE	<i>Asterionella formosa</i> Hansall	ASTERIO
<i>Aphanocapsa delicatissima</i> West & West	APHANO-D	<i>Aulacoseira distans</i> (Ehrenberg) Simonsen	AULACO-D
<i>Aphanocapsa elachista</i> W. & G.S. West	APHANO-E	<i>Aulacoseira</i> sp	AULACO
<i>Aphanizomenon flos-aquae</i> (Linne) Ralfs	APHANI-F	<i>Cyclotella bodanica</i> Grunow	CYCLOT-B
<i>Aphanothece clathrata</i> W & G.S. West	APHANO-C	<i>Cyclotella</i> sp	CYCLOT
<i>Aphanothece</i> sp	APHANO	<i>Cymbella minuta</i> Hilse	CYMBELLA
<i>Chroococcus dispersus</i> (Keissler) Lemmermann	CHROOC-D	<i>Cymatopleura solea</i> (Brebisson) W. Smith	CYMATO

**Table A3-1 continued (2005)**

Identification	Key	Identification	Key
<i>Chroococcus limneticus</i> Lemmermann	CHROOC-L	<i>Diatoma tenuis</i> Agardh	DIATOMA
<i>Gomphosphaeria aponina</i> Kuetzing	GOMPHO-A	<i>Eunotia</i> sp	EUCOTIA
<i>Merismopedia glauca</i> (Ehrenberg) Naegeli	MERIS-G	<i>Fragilaria crotonensis</i> Kitton	FRAGIL-C
<i>Merismopedia tenuissima</i> Lemmermann	MERIS-T	<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	FRAGIL-U
<i>Microcystis aeruginosa</i> Kuetzing	MICRO-A	<i>Gomphonema</i> sp.	GOMPHON
<i>Microcystis ichthyoblabe</i> Kuetzing	MICRO-I	<i>Navicula</i> sp	NAVICULA
<i>Microcystis</i> sp	MICRO	<i>Nitzschia acicularis</i> (Kuetzing) W. Smith	NITZ-A
<i>Oscillatoria limnetica</i> Lemmerman	OSCILL	<i>Nitzschia closterium</i> (Ehrenberg) W. Smith	NITZ-C
<i>Planktolyngya limnetica</i> Lemmermann	PLANKTO-L	<i>Nitzschia</i> sp	NITZ
<i>Pseudanabaena arcuata</i> (Skuja) Anagnostidis & Komarek	PSEUD-A	<i>Pinnularia</i> sp	PINNULAR

**Table A3-1 continued (2005)**

Identification	Key	Identification	Key
<i>Pseudanabaena</i> sp	PSEUD	<i>Rhizosolenia eriensis</i> H.L. Smith	RHIZO-E
<i>Rhabdoderma lineare</i> Woloszynska	RHABDO-L	<i>Rhizosolenia longiseta</i> Ehrenberg	RHIZO-L
<i>Woronichinia compacta</i> (Lemmermann) Komarek	WORON	<i>Stephanodiscus binderanus</i> (Kuetzing) Krieger	STEPHANO
<i>Snowella lacustris</i> (Chodat) Komarek et Hindak	SNOW	<i>Synedra filiformis</i> Grunow	SYNED-F
<i>Synechococcus</i> sp	SYNECH	<i>Synedra radians</i> Kuetzing	SYNED-R
		<i>Synedra</i> sp (cf. <i>S. filiformis</i> + <i>S. radians</i> )	SYNED
<b>CHLOROPHYCEAE</b>		<i>Synedra ulna</i> (Nitzsch) Ehr.	SYNED-U
<i>Ankistrodesmus falcatus</i> var. <i>mirabilis</i> West	ANKIS-FA	<i>Tabellaria flocculosa</i> (Roth) Kuetzing	TABEL-FL
<i>Ankistrodesmus fusiformis</i> Corda	ANKIS-FU		
<i>Ankistrodesmus gracilis</i> (Reinsch) Kors.	ANKIS-G	<b>CHRYSOPHYCEAE</b>	
<i>Ankistrodesmus</i> sp	ANKIS	<i>Bitrichia chodatii</i> (Rev.) Chod.	BRITRICH



**Table A3-1 continued (2005)**

Identification	Key	Identification	Key
<i>Chlamydomonas</i> sp.	CHLAMYD	<i>Chrysocapsella</i> sp	CHRYSOC
<i>Closteriopsis acicularis</i> (G.M. Smith) Belcher & Swale	CLOST-A	<i>Chromulina</i> sp.	CHROMUL
<i>Closteriopsis longissima</i> Lemmermann	CLOST-L	<i>Chrysophaerella longispina</i> Lauterborn	CHRYSOP
<i>Coenocystis</i> sp.	COENOC	<i>Chrysolykos planctonicus</i> Mack	CHRYSOLY
<i>Cosmarium asphaerosporum</i> Nordst	COSMAR-A	<i>Chrysamoeba mikrokonta</i> Skuja	CHRYSAM
<i>Cosmaruim bioculatum</i> Brebisson	COSMAR-B	<i>Codonodendron ocellatum</i> Pascher	CODONO
<i>Cosmarium depressum</i> Nageli (Lund)	COSMAR-D	<i>Dinobryon bavaricum</i> Imhof	DINO-B
<i>Cosmarium phaseolus</i> Brebisson	COSMAR-P	<i>Dinobryon crenulatum</i> W. et. G.S. West	DINO-CR
<i>Cosmarium</i> sp	COSMAR	<i>Dinobryon cylindricum</i> Imhof	CINO-CY
<i>Cosmarium subreniforme</i> Nordstedt	COSMAR-S	<i>Dinobryon dilatatum</i> Hillard	DINO-DL

**Table A3-1 continued (2005)**

<b>Identification</b>	<b>Key</b>	<b>Identification</b>	<b>Key</b>
<i>Crucigenia irregularis</i> Wille	CRUCE-I	<i>Dinobryon divergens</i> Imhof	DINO-DV
<i>Crucegenia quadrata</i> Morren	CRUCE-Q	<i>Dinobryon</i> sp (monad)	DINO
<i>Crucegenia tetrapedia</i> (Kirchner) W. & G.S. West	CRUCE-T	<i>Dinobryon sociale</i> Ehrenberg	DINO-S
<i>Dictyosphaerium pulchellum</i> Skuja	DICTYO	<i>Dinobryon sociale</i> var. <i>americana</i> (Brunthaler) Bachmann	DINO-SA
<i>Elakatothrix gelatinosa</i> Wille	ELAKA-GL	Haptophyceae	HAPTO
<i>Elakatothrix genevensis</i> (Reverdin) Hindak	ELAKA-GU	<i>Kephyrion boreale</i> Skuja	KEPHYR-B
<i>Euastrum</i> sp	EUAST	<i>Kephyrion cupuliforme</i> Conrad	KEPHYR-C
<i>Eutetramorus</i> sp	EUTET	<i>Kephyrion littorale</i> Lund	KEPHYR-L
<i>Gloeocystis planctonica</i> (W. & G.S. West) Lemmermann	GLOE-P	<i>Kephyrion obliquum</i> Hilliard	KEPHYR-O
<i>Gloeocystis</i> sp	GLEOCYST	<i>Mallomonas</i> sp	MALLAMO
<i>Gonium pectorale</i> Mueller	GONIUM	<i>Monosiga</i> sp	MONOSIGA

**Table A3-1 continued (2005)**

Identification	Key	Identification	Key
Miscellaneous microflagellates	MICROFLAG	<i>Ochromonas</i> sp	OCHROMON
<i>Monoraphidium braunii</i> Naegeli	MONORA-B	<i>Pseudokephyrion attenuatum</i> Hilliard	PSEUDO-A
<i>Monoraphidium contortum</i> (Thuret) Komarkova-Legenerova	MONORA-C	<i>Pseudokephyrion ellipsoideum</i> (Pascher) Schmid	PSEUDO-E
<i>Monoraphidium dybowskii</i> (Wolosz) Hindak et. Kom.-Legn.	MONORA-D	<i>Stelexomonas dichotomus</i> Lackey	STELEXOM
<i>Monoraphidium griffithii</i> (Berkeley) Komarkova-Legenerova	MONORA-G	<i>Synura</i> sp	SYNURA
<i>Monoraphidium irregulare</i> (G.M. Smith) Komarkova-Legenerova	MONORA-I		
<i>Monoraphidium minutum</i> (Nag.) Komarkova-Legenerova	MONORA-M	<b>CRYPTOPHYCEAE</b>	
<i>Monoraphidium setiforme</i> Komarkova-Legenerova	MONORA-S	<i>Cryptomonas curvata</i> Ehrenberg	CRYPTOJ-C
<i>Nephrocytium agadhianum</i> Naegeli	NEPHRO-A	<i>Cryptomonas erosa</i> Ehrenberg	CRYPTO-E

**Table A3-1 continued (2005)**

Identification	Key	Identification	Key
<i>Nephrocytium</i> sp	NEPHRO	<i>Cryptomonas marsonii</i> Skuja	CRYPTO-M
<i>Oocystis borgei</i> Snow	OOCYST-B	<i>Cryptomonas phaseolus</i> Skuja	CRYPTO-P
<i>Oocystis gigas</i> Archer	OOCYST-G	<i>Cryptomonas pyrenoidifera</i> Geitler	CRYPTOPY
<i>Oocystis parva</i> W. & G.S. West	OOCYST-P	<i>Cryptomonas reflexa</i> Skuja	CRYPTO-R
<i>Oocystis pusilla</i> Hansgirg	OOCYST-U	<i>Cryptomonas rostratiformis</i> Skuja	CRYPTORO
<i>Oocystis solitaria</i> Wittrock	OOCYST-S	<i>Katablepharis ovalis</i> Skuja	KATABL-O
<i>Oocystis</i> sp	OOCYST	<i>Rhodomonas lens</i> Pascher & Ruttner	RHODO-L
<i>Scenedesmus acutiformis</i> Schroeder	SCENE-AF	<i>Rhodomonas minuta</i> Skuja	RHODO-M
<i>Scenedesmus acutus</i> Meyen	SCENE-AT	<i>Rhodomonas minuta</i> var. <i>nanoplantonica</i> Skuja	RHODO-N
<i>Scenedesmus bicaudatus</i> Dedus	SCENE-BC	<b>DINOPHYCEAE</b>	
<i>Scenedesmus bijuga</i> (Turp.) Lagerheim	SCENE-BJ		
<i>Scenedesmus ecornis</i> (Ehrenberg) Chodat	SCENE-E		
		<i>Amphidinium</i> sp	AMPHID

**Table A3-1 continued (2005)**

Identification	Key	Identification	Key
<i>Scenedesmus incrassatulus</i> Bohlin	SCENE-I	<i>Ceratium hirundinella</i> (O.F. Muller) Schrank	CERATUIM
<i>Scenedesmus quadricauda</i> (Turpin) Brebisson	SCENE-Q	<i>Glenodinium</i> sp	GLENOD
<i>Schroderia setigera</i> (Schroed.) Lemmermann	SCHROD	<i>Gymnodinium ordinatum</i> Skuja	GYMNOD-O
<i>Staurodesmus incus</i> (Brebisson) Teiling	STAURO	<i>Gymnodinium paradoxum</i> Schill.	GYMNOD-P
<i>Pediastrum boryanum</i> (Turpin) Meneghini	PEDIAS	<i>Gymnodinium</i> sp	GYMNOD
<i>Planktonema lauterbornii</i> Schmidle	PLANKONE	<i>Peridinium inconspicuum</i> Lemmermann	PERIDI-I
<i>Tetraedron minimum</i> (A. Braun) Hansgrig	TETRAE-M	<i>Gymnodinium pusillum</i> (Penard) Lemmermann	GYMNOD-L
<i>Tetraedron mimimum</i> var. <i>tetralobulatum</i> Reins	TETRAE-T		
<i>Tetraedron muticum</i> (A. Braun) Hansgrig	TETRAE-U	<b>EUGLENOPHYCEAE</b>	
<i>Treubaria</i> sp	TREUBAR	<i>Euglena polymorpha</i> Dangeard	EUGLEN

**Table A3-1continued (2005)**

<b>Identification</b>	<b>Key</b>	<b>Identification</b>	<b>Key</b>
<i>Zygnema</i> sp	ZYGNEMA	<i>Lepocinclis</i> sp	LEPOC
		<i>Trachelomomas volvocina</i> Ehrenberg	TRACHE-V
		<i>Trachelomomas</i> sp	TRACHE

**Table A.3-2.** Data Matrices of lakes and pits (columns) and species (rows), for all three years, used for multivariate analysis. These data matrices were transposed from those used in multivariate analysis to allow for easier presentation. The species abundances have been transformed (LOG(x+1)), rare and outlying species have been deleted, and outlying lakes have been deleted. These data modifications were necessary for appropriate multivariate analysis.

Species Key	Lakes and Pits Sampled in 2003															
	D-pit- D-pit-				Little Upper				Wolla-							
	A-pit	B-pit	Cluff	Delta C	R	Dubyna	First	Fookes	Fulton	Indigo	Island	Mac	Link	Vulture	ston	Zimmer
ACHNAN	0.000	0.000	0.000	0.000	4.571	0.000	4.419	0.000	0.000	4.680	4.884	0.000	0.000	0.000	0.000	0.000
ANABAE-S	0.000	0.000	4.253	0.000	0.000	0.000	5.279	4.941	4.975	3.733	0.000	0.000	0.000	0.000	0.000	4.190
ANABAE	0.000	0.000	0.000	0.000	0.000	0.000	4.051	0.000	0.000	5.044	3.994	0.000	4.369	0.000	0.000	4.180
ANKIST-F	0.000	0.000	2.149	0.000	0.000	0.000	0.000	0.000	3.927	0.000	3.688	0.000	0.000	0.000	3.654	0.000
APHANO	0.000	0.000	3.982	0.000	0.000	0.000	0.000	4.884	4.491	3.927	0.000	0.000	3.865	0.000	0.000	0.000
APHATH-N	0.000	0.000	3.927	0.000	0.000	0.000	4.954	4.815	3.575	3.927	0.000	0.000	0.000	0.000	0.000	4.653
ASTERIO	4.896	0.000	0.000	0.000	0.000	0.000	3.747	3.595	0.000	3.802	2.973	0.000	2.450	4.655	0.000	3.704
ATHROD-I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.371	0.000	0.000	3.654	0.000	0.000	0.000
BRITRICH	3.848	0.000	4.213	0.000	4.686	0.000	4.419	4.583	4.052	3.672	4.404	0.000	3.052	0.000	0.000	4.197
BOTRYOC	0.000	0.000	2.626	0.000	2.530	0.000	2.972	3.750	3.973	3.052	0.000	3.052	2.405	0.000	2.672	3.052
CERATIUM	3.024	0.000	0.000	0.000	2.597	0.000	2.274	2.955	1.973	0.000	2.973	0.000	2.053	4.156	0.000	2.354
CHLAMID	0.000	4.865	0.000	3.818	0.000	4.883	0.000	0.000	0.000	0.000	5.131	0.000	0.000	5.387	4.915	0.000
CHROOC-M	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.876	0.000	0.000	4.352	2.575	0.000	0.000	0.000
CHROOC	0.000	0.000	0.000	0.000	0.000	0.000	5.118	3.750	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.307
CHRYSO LY	0.000	0.000	4.228	0.000	0.000	0.000	0.000	4.750	0.000	3.672	0.000	0.000	3.353	0.000	0.000	0.000
CHRYSO SP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.423	0.000	5.417	0.000	0.000	0.000
CILIA TE1	2.061	3.341	2.992	2.992	0.000	3.919	3.124	3.887	2.947	2.281	3.418	2.061	3.055	2.786	3.680	3.351
CILIA TE2	0.000	0.000	0.000	0.000	0.000	3.230	0.000	0.000	0.000	0.000	2.155	0.000	1.826	0.000	0.000	0.000
CRYPTO-E	0.000	3.786	4.473	0.000	4.507	5.356	4.148	4.491	3.862	3.371	4.052	0.000	0.000	4.584	5.055	2.274
CRYPTO-M	0.000	0.000	4.342	4.081	5.190	5.194	4.227	4.394	4.767	3.325	4.491	0.000	0.000	5.468	5.019	0.000
CRYPTO-R	0.000	0.000	0.000	0.000	0.000	3.826	2.875	0.000	0.000	0.000	0.000	0.000	0.000	4.831	3.564	0.000
CYCLOT-K	0.000	0.000	4.571	0.000	0.000	0.000	3.954	0.000	4.824	4.203	4.792	0.000	0.000	0.000	6.286	0.000
CYCLOT-M	0.000	0.000	5.341	0.000	0.000	5.479	5.233	0.000	0.000	0.000	0.000	0.000	3.353	0.000	0.000	5.277
CYCLOT	0.000	0.000	3.263	0.000	0.000	0.000	0.000	0.000	3.751	3.769	0.000	5.068	0.000	0.000	0.000	3.449
DAPHNIA	0.000	0.000	0.000	1.301	0.000	2.577	0.000	0.000	0.000	1.301	0.000	0.000	0.000	0.000	0.000	0.000

Table A3-2 continued (2003)

Species Key	A-pit	B-pit	Cluff	Delta	C	D-pit- R	D-pit- R	Dubyna	First	Fookes	Fulton	Indigo	Island	Mac	Little	Upper	Vulture	Wolla- ston	Zimmer
DINO-B	0.000	0.000	3.654	0.000	4.364	0.000	0.000	0.000	4.072	0.000	0.000	5.234	0.000	3.758	0.000	0.000	0.000	2.927	4.282
DINO-D	3.769	4.103	0.000	0.000	5.219	0.000	4.615	0.000	4.799	0.000	0.000	4.295	0.000	4.915	5.433	0.000	0.000	4.136	0.000
EPIPYXIS	0.000	0.000	4.715	0.000	0.000	0.000	0.000	0.000	4.784	0.000	0.000	0.000	0.000	3.955	0.000	0.000	0.000	0.000	0.000
EUDOR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.353	3.831	3.830	0.000	0.000	0.000
EUGLEN	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.371	0.000	0.000	3.354	3.052	0.000	0.000	0.000
GEITLERI	0.000	0.000	4.149	0.000	0.000	0.000	6.800	0.000	0.000	0.000	0.000	0.000	4.714	0.000	0.000	3.751	0.000	0.000	0.000
GLENOD	4.450	4.596	0.000	3.894	0.000	0.000	0.000	0.000	4.431	3.575	0.000	4.190	0.000	0.000	5.699	4.599	3.596	4.148	0.000
GLEOCYST	0.000	0.000	0.000	0.000	0.000	0.000	4.352	0.000	4.112	3.927	0.000	0.000	4.844	4.715	0.000	3.529	0.000	3.653	0.000
GYMNOD1	0.000	0.000	1.672	0.000	4.499	0.000	0.000	0.000	2.053	0.000	0.000	0.000	0.000	0.000	0.000	4.844	2.450	0.000	0.000
GYMNOD2	3.246	0.000	2.450	0.000	3.214	3.747	0.000	0.000	3.006	0.000	0.000	3.575	0.000	2.297	0.000	0.000	2.149	2.751	0.000
GYROMI-C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.751	4.353	0.000	3.353	4.558	0.000	2.994	3.052	0.000
KATABL-O	0.000	0.000	4.808	0.000	0.000	5.525	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.742	0.000	0.000	0.000
KATABL	0.000	0.000	0.000	0.000	4.952	0.000	5.344	0.000	5.402	0.000	4.654	5.276	0.000	4.590	5.512	0.000	4.166	4.943	0.000
KELLICOT	1.301	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.462	0.000	0.000	1.462	0.000	0.000	0.000	0.000	0.000
KEPHYR	0.000	0.000	4.414	0.000	0.000	0.000	0.000	0.000	4.750	0.000	0.000	5.030	0.000	3.654	0.000	0.000	4.374	4.197	0.000
KERATELA	1.000	0.000	0.000	0.000	0.000	2.878	0.000	0.000	2.824	0.000	0.000	0.000	3.043	1.301	0.000	0.000	0.000	1.887	0.000
LYNGBYA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.123	0.000	0.000	0.000	0.000	0.000	0.000	3.897	0.000	5.266	0.000
MALLOMO	0.000	0.000	2.927	0.000	0.000	0.000	3.875	0.000	0.000	0.000	0.000	4.654	0.000	0.000	5.146	0.000	2.848	0.000	0.000
MERIS-M	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.319	0.000	4.242	0.000	0.000	0.000	4.631	0.000
MONORA	0.000	0.000	3.379	0.000	4.557	5.194	0.000	0.000	0.000	4.683	5.468	4.848	0.000	4.414	0.000	4.131	0.000	5.440	0.000
MOUGEO-S	0.000	4.103	2.371	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.449	0.000
NAUPLIUS	0.000	0.000	1.000	1.000	0.000	2.754	0.000	0.000	0.000	1.301	0.000	0.000	0.000	1.681	0.000	1.301	1.000	1.591	0.000
NAVICULA	0.000	4.792	0.000	3.973	0.000	0.000	3.574	0.000	0.000	0.000	0.000	0.000	5.612	0.000	0.000	3.955	0.000	0.000	0.000
NITZ-S	0.000	4.450	0.000	3.738	0.000	4.349	0.000	0.000	0.000	0.000	0.000	0.000	4.733	0.000	0.000	0.000	0.000	0.000	0.000
OOCYST-L	0.000	0.000	0.000	0.000	0.000	0.000	3.926	0.000	4.784	0.000	4.252	4.529	0.000	0.000	0.000	4.006	0.000	0.000	0.000
PERIDI-I	0.000	4.344	3.353	0.000	4.459	4.446	0.000	0.000	4.384	0.000	0.000	0.000	0.000	2.876	0.000	3.812	3.228	0.000	0.000
PERDI	0.000	3.070	2.149	0.000	0.000	0.000	0.000	0.000	2.751	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
POLYART	1.681	2.633	2.571	2.571	2.824	3.480	0.000	0.000	1.982	1.301	1.462	2.155	0.000	2.061	0.000	3.225	2.260	0.000	0.000
PSEUD-S	0.000	6.004	4.166	0.000	0.000	0.000	4.051	0.000	0.000	0.000	0.000	0.000	4.714	0.000	0.000	4.283	0.000	0.000	0.000
QUADRIG	0.000	0.000	0.000	0.000	0.000	0.000	4.858	0.000	4.844	4.177	0.000	4.228	0.000	2.751	0.000	0.000	0.000	0.000	0.000
RHABDO-L	0.000	0.000	4.073	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.507	0.000	0.000	0.000	5.024	0.000



Table A3-2 continued (2003)

Species Key	A-pit	B-pit	Cluff	Delta	C	D-pit- R	Dubyna	First	Fookes	Fulton	Indigo	Island	Mac	Little	Upper	Wolla- Vulture	ston	Zimmer
RHIZOS-E	0.000	0.000	5.186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.955	0.000	0.000	0.000	0.000	0.000	4.319	5.178
RHODO-L	0.000	0.000	4.432	0.000	4.987	0.000	5.255	5.261	0.000	5.080	5.073	0.000	0.000	0.000	0.000	0.000	5.075	0.000
RHODO-N	0.000	0.000	4.602	0.000	0.000	0.000	0.000	5.277	5.633	0.000	0.000	0.000	0.000	0.000	5.530	0.000	0.000	0.000
RHOPA	0.000	0.000	1.672	0.000	0.000	0.000	2.274	0.000	1.973	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ROTIFERS	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.826	2.997	1.301	0.000	2.061	0.000
ROYA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.255	0.000	4.252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.920
SPONDYL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.973	3.626	0.000	2.672	0.000	0.000	0.000	0.000	3.528
SYNED-L	0.000	0.000	4.942	0.000	0.000	6.451	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.470	2.903	0.000	0.000
SYNED-M	4.111	0.000	0.000	0.000	4.006	4.826	0.000	4.148	3.450	0.000	4.626	0.000	0.000	0.000	2.927	0.000	0.000	0.000
SYNED-U	0.000	2.672	0.000	0.000	0.000	3.224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.274	0.000	0.000	0.000
TABEL-FE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.371	0.000	3.070	0.000	2.450	0.000	0.000	3.315	3.528	0.000
TABEL-FL	0.000	0.000	3.404	3.040	3.450	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.818	0.000	0.000	0.000	0.000	0.000
TETRAED	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.307	4.252	0.000	0.000	5.060	0.000	0.000	0.000	0.000	0.000	0.000

## Lakes and Pits Sampled in 2004

Species Keys	A Pit	B-pit	D-pit-R	Upper Link	Vulture	Sue	C Island	D-pit-C	Ace	Dubyna	Fulton	Fredette	ston	Cluff	First
ANABAE-S	5.4490	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.947	4.694	5.291	4.520	4.210	3.815	5.478
APHANO	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.646	4.789	4.152	0.000	0.000	0.000	0.000
APHATH-N	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.549	0.000	3.784	5.070	0.000	0.000	0.000
ASTERIO	5.4552	5.054	1.98	4.497	0.000	0.000	0.000	0.000	3.345	0.000	4.047	4.361	5.024	0.000	0.000
BRITRICH	0.000	0.000	0.000	0.000	4.986	0.000	0.000	4.324	3.149	3.897	4.006	3.784	0.000	4.535	3.981
BOSMINA	0.000	0.000	2.281	1.462	0.000	0.000	0.000	0.000	0.602	0.000	0.000	0.000	0.000	0.000	0.000
BOTRYOC	2.2830	0.000	0.000	0.000	0.000	0.000	2.943	0.000	3.228	3.499	2.909	3.851	0.000	2.725	2.760
CERATIUM	2.5840	0.000	3.119	3.379	0.000	0.000	0.000	2.760	1.908	1.602	0.000	2.785	0.000	1.613	2.885

**Table A3-2 continued (2004)**

Species Keys	Upper			Wolla-				Cluff	First				
	A Pit	B-pit	D-pit-RLink	Vulture	Sue C Island	D-pit-CAce	Dubyna			Fulton	Fredette	ston	
CHLAMID	0.000	0.000	0.000	0.000	7.084	4.465	0.000	0.000	0.000	4.348	0.000	0.000	0.000
CHROOC-M	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.207	4.736	3.608	3.570	0.000	0.000
CILIATE1	2.582	2.236	3.093	3.108	2.760	2.455	1.763	0.000	2.196	2.743	2.757	1.833	2.914
CILIATE-	2.687	1.833	2.127	2.236	2.161	0.000	1.301	0.000	2.188	0.000	0.000	0.000	0.000
CRYPTO-E	0.000	3.759	5.035	4.907	5.056	0.000	0.000	4.517	2.751	3.581	3.307	4.269	4.016
CRYPTO-S	0.000	4.538	5.337	5.224	5.098	5.295	0.000	0.000	4.321	4.011	0.000	0.000	4.192
CYCLOT	0.000	0.000	0.000	0.000	0.000	0.000	4.192	0.000	3.383	0.000	3.976	3.006	0.000
DINO-BA	4.396	2.505	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.759	0.000
DINO-BO	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.286	0.000	4.152	0.000	0.000
DINO-D	0.000	2.806	4.675	0.000	0.000	0.000	0.000	0.000	3.258	3.835	0.000	4.172	4.155
GEITLER	0.000	0.000	0.000	0.000	0.000	6.213	5.229	0.000	0.000	0.000	0.000	4.085	0.000
GLENOD	4.095	3.203	4.675	5.055	0.000	0.000	4.954	3.759	3.751	0.000	0.000	0.000	3.414
GYMNOD	2.981	4.127	4.585	0.000	3.579	0.000	0.000	0.000	0.000	0.000	0.000	2.529	2.512
KATABL	0.000	4.583	4.710	5.175	0.000	0.000	4.891	0.000	4.424	4.784	4.386	4.784	0.000
KELLICOT	1.041	0.000	2.185	0.000	0.000	0.000	0.000	0.000	0.602	1.301	1.041	1.477	1.591
KEPHYR	4.428	0.000	5.441	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.952
KERATELA	0.000	0.000	0.000	0.000	2.839	2.790	1.833	0.000	0.602	0.000	1.301	0.000	1.301
LIMNOCA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.602	1.690	1.041	0.000	1.591
MONORA	0.000	0.000	0.000	4.622	5.135	0.000	5.649	0.000	4.804	4.488	4.537	4.649	0.000
NAUPLIUS	1.591	0.000	1.301	1.763	0.000	0.000	2.025	0.000	0.845	1.477	0.000	0.000	1.982
NITZ	0.000	3.902	0.000	4.476	0.000	0.000	0.000	0.000	3.809	3.675	0.000	0.000	0.000
OOCYST-L	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.161	4.531	0.000	4.307	0.000
POLYART	2.281	1.763	2.486	2.886	0.000	2.977	0.000	0.000	1.954	0.000	1.041	0.000	1.591
PSEUD-S	5.299	5.856	5.758	0.000	0.000	0.000	5.102	0.000	3.947	0.000	0.000	2.830	4.369
QUADRIG	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.374	0.000	3.909	0.000
RHOPA	2.283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.908	0.000	2.009	0.000	0.000

**Table A3-2 continued (2004)**

Species Keys	Upper					First										
	A Pit	B-pit	D-pit-R	Link	Vulture Sue	C Island	D-pit-C	Ace	Dubyna	Fulton	Fredette	Wollaston	Cluff	First		
ROTIFERS	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.833	1.708	1.690	1.982	2.025	0.000	2.083	2.127	
ROYA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.742	4.006	4.210	0.000	0.000	0.000	
SYNED-L	2.760	2.806	4.970	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.006	0.000	0.000	0.000	0.000	
SYNED-M	4.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.006	3.608	3.854	0.000	0.000	
TABEL-FL	0.000	0.000	0.000	0.000	3.652	3.278	0.000	0.000	0.000	3.646	0.000	3.006	4.142	3.511	0.000	0.000
TEMNOGA	0.000	3.990	0.000	0.000	0.000	0.000	0.000	4.833	0.000	0.000	0.000	0.000	0.000	2.436	0.000	
TETRAE-M	0.000	0.000	5.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.608	0.000	0.000	0.000	4.127	

**Lakes and Pits Sampled in 2005**

<b>Species Key</b>	B-pit	D-pit-R	Upper Link	McClean	Cluff	Island	D-Pit-C	Ace	Dubyna	Fookes	Beaver	Delta	Gaertner	Little Mac	Vulture	Wolla- ston	Indigo	First	Fredette	Zimmer
ANABAE-F	0.000	0.000	3.879	0.000	3.578	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.754	0.000
APHANO-E	0.000	0.000	0.000	0.000	4.481	0.000	0.000	4.231	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.453	4.958	4.277
APHANI-F	0.000	0.000	4.833	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.833	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.532	3.879
APHANO-C	0.000	0.000	0.000	0.000	4.481	0.000	0.000	5.662	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.532	4.453	5.502	0.000
CHROOC-L	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.754	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.532	4.231	0.000	3.578
MERIS-T	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.754	3.407	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.578
MICRO-I	0.000	0.000	0.000	0.000	3.578	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.055	0.000	0.000
MICRO	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.963	0.000	0.000	3.833	0.000	0.000	0.000	0.000	0.000	3.833	0.000	0.000	0.000
OSCILL	5.025	0.000	0.000	3.879	3.578	0.000	0.000	3.833	4.356	0.000	3.407	0.000	0.000	0.000	0.000	4.134	0.000	4.055	0.000	5.025
PLANKTO-	0.000	0.000	0.000	0.000	3.879	0.000	0.000	0.000	3.754	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.754	0.000	0.000
PSEUD-A	0.000	5.532	0.000	0.000	0.000	4.453	0.000	0.000	0.000	0.000	0.000	6.839	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RHABDO-L	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.231	0.000	0.000	0.000	0.000	4.055	0.000	0.000	0.000	0.000	0.000	3.754	3.578

**Table A3-2 continued (2005)**

Species	Key B-pit	D-pit-R	Upper Link	McClean Cluff	Island D-Pit-C	Ace	Dubyna	Fookes	Beaver	Delta	Gaertner	Little Mac	Vulture	Wolla-ston	Indigo First	Fredette	Zimmer
SNOW	0.000 0.000	0.000 0.000	0.000 0.000	0.000 0.000 0.000	3.833 0.000	3.754	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	3.754	3.578	
SYNECH	0.000 0.000	0.000 0.000	0.000 0.000	3.879 4.453 0.000	4.134 0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	4.180	0.000	0.000 0.000 0.000	0.000	3.879	
ANKIS-FU	0.000 4.055	0.000 0.000	0.000 0.000	0.000 0.000 0.000	0.000 0.000	4.231	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	4.782	0.000	0.000 0.000 0.000	0.000	4.754	
CHLAMYD	3.578 0.000	0.000 0.000	0.000 0.000	0.000 4.055 0.000	0.000 3.754	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	0.000	4.310	0.000 0.000 0.000	0.000	0.000	
CRUCE-Q	0.000 0.000	0.000 3.879	0.000 0.000	0.000 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 3.833 0.000	3.754	0.000	
CRUCE-T	0.000 0.000	0.000 3.578	0.000 0.000	0.000 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	4.277	0.000	0.000 0.000 0.000	0.000	0.000	
ELAKA-GU	0.000 0.000	0.000 0.000	0.000 0.000	0.000 3.754 0.000	4.310 4.453	3.754	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	4.055	0.000	0.000 4.009 0.000	4.055	3.879	
GLOE-P	0.000 0.000	3.879 0.000	0.000 0.000	3.578 3.754 0.000	0.000 0.000	4.657	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	4.277	0.000	3.532 0.000 0.000	3.754	4.055	
GLEOCYST	0.000 0.000	0.000 0.000	0.000 0.000	0.000 4.055 3.453	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	3.754	3.578	
MONORA-B	0.000 0.000	0.000 0.000	0.000 0.000	3.578 0.000 0.000	3.833 4.055	3.754	3.708	3.532 4.055	0.000	0.000 0.000	0.000	0.000	4.310	0.000 0.000 0.000	3.754	3.578	
MONORA-C	0.000 0.000	0.000 3.578	0.000 0.000	0.000 4.708 0.000	3.532 0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	0.000	4.310	0.000 0.000 0.000	0.000	0.000	
MONORA-D	0.000 4.532	0.000 0.000	0.000 0.000	0.000 0.000 0.000	4.231 4.231	4.868	0.000	4.134 0.000	0.000	0.000 0.000	0.000	3.879	0.000	0.000 0.000 0.000	4.231	0.000	
MONORA-I	0.000 0.000	0.000 0.000	0.000 0.000	0.000 0.000 0.000	3.532 4.055	0.000	3.884	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000	
MONORA-M	0.000 0.000	0.000 0.000	0.000 0.000	0.000 0.000 3.453	4.231 5.152	0.000	0.000	0.000 0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	3.754	0.000	
MONORA-S	0.000 3.754	0.000 0.000	0.000 0.000	0.000 0.000 0.000	3.833 0.000	0.000	4.252	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 3.532 0.000	0.000	0.000	
OOCYST-B	0.000 0.000	0.000 0.000	0.000 0.000	0.000 4.532 0.000	0.000 3.754	0.000	0.000	3.833 0.000	0.000	0.000 0.000	0.000	3.578	0.000	0.000 0.000 0.000	4.055	3.578	
OOCYST-P	0.000 0.000	0.000 0.000	0.000 0.000	0.000 4.930 0.000	3.532 0.000	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	4.055	4.310	0.000 0.000 0.000	4.231	4.277	
OOCYST-U	0.000 0.000	0.000 0.000	0.000 0.000	3.879 4.754 0.000	0.000 3.754	3.754	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	4.231	0.000	
OOCYST-S	0.000 0.000	0.000 0.000	0.000 0.000	0.000 0.000 0.000	0.000 3.754	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	0.000	3.578	
OOCYST	0.000 0.000	0.000 4.055	0.000 0.000	0.000 0.000 0.000	3.833 0.000	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	0.000	3.578	
SCENE-BJ	0.000 0.000	3.578 0.000	0.000 0.000	0.000 5.033 0.000	0.000 3.754	3.754	3.884	3.833 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 3.532 0.000	0.000	0.000	
TETRAE-M	0.000 4.900	0.000 3.578	0.000 3.578	0.000 0.000 0.000	0.000 0.000	4.055	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	3.754	0.000	
TETRAE-T	0.000 5.134	0.000 0.000	0.000 0.000	4.055 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	3.833 0.000 0.000	0.000	0.000	
ACHNAN-M	3.578 0.000	0.000 3.578	0.000 3.578	0.000 5.033 3.453	0.000 3.754	4.356	0.000	3.532 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	3.754	0.000	
ASTERIO	0.000 5.494	3.879 3.578	0.000 0.000	0.000 3.754 0.000	4.134 0.000	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	4.787	4.377 4.231 0.000	4.356	0.000	
AULACO	0.000 0.000	0.000 0.000	0.000 0.000	0.000 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	3.532 0.000 0.000	4.231	3.578	
CYCLOT-B	0.000 0.000	0.000 0.000	0.000 0.000	0.000 0.000 0.000	3.532 4.356	0.000	0.000	0.000 0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000 0.000	4.231	3.578	

Table A3-2 continued (2005)

Species Key	B-pit	D-pit-R	Upper Link	McClean Cluff	Island D-Pit-C	Ace	Dubyna	Fookes	Beaver	Delta	Gaertner	Little Mac	Vulture	Wollaston	Indigo First	Fredette	Zimmer
CYCLOT	0.000 0.000	4.277	0.000	4.754 4.055 0.000	4.231 4.231	4.055	0.000	4.787	4.055	0.000	4.611	4.736	3.833	4.231 0.000	0.000	0.000	0.000
GOMPHON	0.000 0.000	0.000	0.000	0.000 0.000 3.754	3.532 0.000	0.000	0.000	3.833	0.000	0.000	0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000	0.000
NAVICULA	3.578 0.000	0.000	3.578	0.000 4.055 0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000	0.000 0.000 0.000	0.000	0.000	0.000
NITZ	0.000 0.000	0.000	0.000	0.000 3.754 0.000	0.000 0.000	0.000	0.000	0.000	0.000 0.000	0.000	4.611	3.833	4.009	0.000 0.000	0.000	0.000	0.000
RHIZO-L	0.000 0.000	0.000	3.879	4.692 0.000 0.000	0.000 3.754	0.000	4.252	0.000 0.000	0.000	0.000	0.000	4.009	3.833	4.599 0.000	4.724		
SYNED	0.000 4.657	0.000	4.356	4.055 4.356 0.000	0.000 4.231	0.000	4.310	4.134 0.000	0.000	0.000	0.000	4.134	4.310	4.356 4.356	5.341		
BRITRICH	0.000 0.000	0.000	3.578	3.879 0.000 3.453	4.009 0.000	0.000	3.407	0.000 0.000	0.000	0.000	0.000	3.578	0.000	3.833 3.754 0.000	0.000	0.000	0.000
DINO-B	0.000 4.231	0.000	4.277	0.000 0.000 4.567	3.833 0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	4.231	0.000	4.231 3.754	3.578		
DINO-DL	4.578 0.000	0.000	3.879	3.578 0.000 0.000	0.000 4.657	0.000	3.884	0.000 0.000	0.000	0.000	0.000	0.000	4.310	4.055 0.000	3.578		
DINO-DV	0.000 0.000	0.000	0.000	0.000 0.000 0.000	4.435 3.754	0.000	0.000	0.000 0.000	0.000	0.000	0.000	3.833	0.000	4.356 4.599	0.000	0.000	0.000
DINO	4.692 0.000	0.000	4.180	3.578 0.000 3.930	4.009 4.795	0.000	0.000	0.000 0.000	0.000	0.000	4.611	0.000	4.009	0.000 0.000	0.000	0.000	0.000
DINO-S	3.879 3.754	0.000	3.879	0.000 0.000 0.000	4.009 0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	4.134	3.833	3.754 0.000	0.000	0.000	0.000
DINO-SA	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000 4.657	0.000	0.000	0.000 0.000	0.000	0.000	5.351	0.000	0.000	3.754 0.000	0.000	0.000	0.000
HAPTO	0.000 0.000	0.000	0.000	0.000 0.000 0.000	3.532 0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	3.532	0.000	4.453 3.754	4.578		
MALLAMO	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	3.532	3.833	0.000 4.599	0.000	0.000	0.000
MONOSIGA	0.000 4.708	0.000	0.000	0.000 0.000 0.000	3.833 0.000	0.000	3.708	0.000 0.000	0.000	0.000	0.000	0.000	0.000	0.000 3.754	0.000	0.000	0.000
SYNURA	0.000 3.754	0.000	0.000	0.000 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000	0.000	0.000	4.611	0.000	3.532	0.000 0.000	0.000	0.000	0.000
CRYPTO-E	0.000 0.000	3.879	3.578	3.879 0.000 0.000	3.532 0.000	3.754	0.000	0.000 0.000	0.000	0.000	0.000	3.532	0.000	4.055 0.000	0.000	0.000	0.000
CRYPTO-M	0.000 4.795	5.391	0.000	4.055 0.000 4.152	0.000 0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	3.879	0.000	0.000 0.000	3.879		
CRYPTO-PY	0.000 0.000	3.578	0.000	0.000 0.000 0.000	3.532 0.000	0.000	0.000	0.000 0.000	0.000	0.000	4.310	0.000	0.000	0.000 0.000	0.000	0.000	0.000
CRYPTO-R	0.000 4.356	4.782	3.578	4.055 4.055 3.930	0.000 0.000	3.754	0.000	0.000 0.000	0.000	0.000	4.787	0.000	3.532	4.055 4.356	0.000	0.000	0.000
RHODO-L	0.000 4.055	0.000	0.000	0.000 0.000 0.000	0.000 0.000	0.000	0.000	0.000 0.000	0.000	0.000	4.611	0.000	0.000	0.000 0.000	0.000	0.000	0.000
GYMNOD-O	4.481 4.055	0.000	4.578	3.578 0.000 0.000	0.000 4.356	3.754	3.884	4.435 0.000	0.000	0.000	0.000	3.578	0.000	3.754 0.000	3.879		
GYMNOD	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000 3.754	4.055	3.407	0.000 0.000	0.000	0.000	4.611	0.000	4.134	0.000 0.000	0.000	0.000	0.000
PERIDI-I	0.000 5.185	0.000	0.000	0.000 0.000 0.000	3.532 0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000	0.000	0.000	0.000 0.000	0.000	0.000	0.000
GYMNOD-L	0.000 0.000	0.000	0.000	0.000 0.000 0.000	0.000 0.000	0.000	3.407	0.000 0.000	0.000	0.000	5.009	0.000	0.000	3.754 0.000	0.000	0.000	0.000

## APPENDIX 4. Biodiversity Summary Statistics

**Table A4-1.** Summary statistics of the species matrices for all years (2003-2005). Outlying lakes, outlying species, and rare species were deleted from the species matrices, for all years, prior to summary analysis. Richness represents species richness in lakes, Evenness represents the evenness of species distribution in lakes, D' represents Simpson's Diversity Index or the probability that two species pulled out of a lake at random will be different.

2003				2004				2005			
Lake	Richness	Evenness	D'	Name	Richness	Evenness	D'	Name	Richness	Evenness	D'
A-pit	11	0.960	0.8931	A-pit	17	0.971	0.9317	B-pit	8	0.996	0.8728
B-pit	13	0.990	0.9191	B-pit	15	0.980	0.9259	D-pit-rabbit	16	0.997	0.9364
Cluff	35	0.986	0.9688	D-pit-Rabbit	18	0.978	0.9380	Upper Link	9	0.995	0.8865
Delta	10	0.969	0.8879	Upper Link	14	0.977	0.9206	McLean	17	0.999	0.9408
D-pit-Cluff	17	0.992	0.9386	Vulture	9	0.980	0.8795	Cluff	18	0.999	0.9440
D-pit-Rabbit	21	0.988	0.9490	Sue C	6	0.954	0.8060	Island	16	0.998	0.9368
Dubyna	24	0.992	0.9564	Island	13	0.965	0.9109	D-pit-Cluff	9	0.998	0.8878
First	33	0.993	0.9683	D-pit-Cluff	5	0.971	0.7828	Ace	29	0.999	0.9653
Fookes	24	0.983	0.9544	Ace	30	0.964	0.9697	Dubyna	24	0.998	0.9579
Fulton	24	0.983	0.9543	Dubyna	20	0.977	0.9439	Fookes	19	0.998	0.9466
Indigo	31	0.992	0.9661	Fulton	24	0.975	0.9526	Beaverlodge	13	0.999	0.9226
Island	13	0.989	0.9190	Fredette	25	0.989	0.9574	Delta	9	0.999	0.8884
Little McDonald	35	0.984	0.9683	Wollaston	17	0.973	0.9329	Gaertner	2	0.952	0.4673
Upper Link	16	0.992	0.9350	Cluff	13	0.981	0.9159	Little McDonald	15	0.999	0.9328
Vulture	27	0.986	0.9598	First	15	0.984	0.9282	Vulture	14	0.999	0.9283
Wollaston	29	0.986	0.9625					Wollaston	15	0.999	0.9329
Zimmer	29	0.988	0.9629					Indigo	20	0.999	0.9496
								First	25	0.999	0.9598
								Fredette	19	0.998	0.9466
								Zimmer	24	0.998	0.9578
Average	23.1	0.985	0.9449	Average	16.1	0.975	0.9131	Average	16	0.996	0.9081
Avg. Skewness	1.124			Avg. Skewness	1.062			Avg. Skewness	1.390		
Avg. Kurtosis	0.165			Avg. Kurtosis	0.464			Avg. Kurtosis	1.700		

## APPENDIX 5. Phosphorus cycling and respiration measurements

**Table A5-1.** Phosphorus cycling measurements for reference lakes and pits. Measurements included are regeneration rate (REG (nM day<sup>-1</sup>)), and turnover rate (TVR (% day<sup>-1</sup>)), and turnover time of phosphate (PO<sub>4</sub>TT (min.)).

Lake	Sampling Year	REG (nM day <sup>-1</sup> )	TVR (% day <sup>-1</sup> )	PO <sub>4</sub> TT (min)
Fulton	2003	35.396	23.216	2.865
Zimmer	2003	8.634	10.615	6.126
Reindeer	2003	41.331	24.845	4.297
Lac La Ronge	2003	84.963	30.663	8.525
Wollaston	2003	26.080	34.967	5.167
First	2003	32.128	17.256	6.073
Fulton	2004	52.549	30.679	2.872
Fredette	2004	41.204	19.531	3.958
Wollaston	2004	29.862	15.327	6.776
McLean	2004	39.299	21.716	5.804
Cluff	2004	23.929	34.184	6.414
First	2004	58.420	29.163	3.370
Wollaston	2005	12.009	11.371	6.485
Indigo	2005	26.762	19.240	5.932
First	2005	23.310	13.937	6.211
Fredette	2005	26.914	8.184	3.445
Zimmer	2005	19.259	10.991	8.802

**Table A5-2.** Phosphorus cycling measurements for exposed lakes and pits. Measurements included are regeneration rate (REG (nM day<sup>-1</sup>)), and turnover rate (TVR (% day<sup>-1</sup>)), and turnover time of phosphate (PO<sub>4</sub>TT (min.)).

Lake	Sampling Year	Reg (nM day <sup>-1</sup> )	TVR (% day <sup>-1</sup> )	PO <sub>4</sub> TT (min)
Dubyna	2003	43.535	31.220	3.549
Fookes	2003	22.278	23.421	4.482
Little Mac	2003	13.130	8.565	6.308
Lower Delta	2003	5.124	7.282	6.027
Upper Link	2003	471.313	40.070	7.503
A Pit	2003	34.202	34.288	4.380
D Pit (Rabbit)	2003	73.608	24.338	3.976
B Pit	2003	51.378	50.595	15.720
D Pit (Cluff)	2003	21.402	19.246	5.983
Island	2003	40.533	25.866	11.593
Cluff	2003	10.269	33.125	5.016
Vulture	2003	34.257	9.092	4.230
Indigo	2003	26.632	18.537	5.419
Sue-C Pit	2003	152.866	15.670	17.473
A Pit	2004	18.506	15.177	5.607
B-pit	2004	22.971	33.431	20.054
D-pit(rabbit)	2004	65.354	17.632	3.577
Upper Link	2004	197.919	28.418	44.685
Indigo	2004	24.029	27.487	4.943
Vulture	2004	51.820	24.084	4.354
Sue C	2004	85.480	8.020	6.308
DJX-pit	2004	8.428	4.154	18.461
Island	2004	5.228	2.605	2.647
D-pit(Cluff)	2004	44.575	30.504	10.669
Ace	2004	30.369	22.522	3.284
Dubyna	2004	62.343	28.802	3.840
Fookes	2004	32.380	19.995	4.033
B-pit	2005	31.653	21.556	9.605
D-pit(rabbit)	2005	61.277	20.859	3.781
Upper Link	2005	253.302	41.302	36.701
Mclean	2005	27.570	31.353	2.175
Cluff	2005	8.393	13.773	6.923
Island	2005	28.433	16.191	5.040
D-pit(Cluff)	2005	29.411	13.762	4.013
Ace	2005	12.935	12.582	11.662
Dubyna	2005	29.070	26.765	3.151
Fookes	2005	14.195	12.220	6.077
Beaverlodge	2005	5.498	8.822	4.673
Delta	2005	9.221	9.200	4.393
Gaertner pit	2005	20.397	18.685	13.890
Little McDonald	2005	12.036	11.238	6.102
Vulture	2005	41.632	15.373	3.736



**Table A5-3.** Planktonic respiration ( $\text{O}_2$  consumed in  $\mu\text{g L}^{-1} \text{day}^{-1}$ ) and total phosphorus (TP in  $\mu\text{g L}^{-1}$ ) measurements for each lake and pit sampled in 2005.

Lake	Lake Condition	TP ( $\mu\text{g L}^{-1}$ )	$\text{O}_2$ Consumed ( $\mu\text{g L}^{-1} \text{d}^{-1}$ )
B-pit	exposed	9.865	57.067
D-pit(Rabbit)	exposed	14.259	170.133
Upper Link	exposed	46.221	143.467
Mclean	exposed	7.556	135.467
Vulture	exposed	12.259	123.733
D-pit(Cluff)	exposed	11.616	32.533
Cluff	exposed	4.143	28.800
Island	exposed	9.630	42.667
Ace	exposed	5.441	38.400
Dubyna	exposed	11.578	70.933
Fookes	exposed	5.694	55.467
Beaverlodge	exposed	3.381	52.800
Delta	exposed	6.166	17.600
Gaertner pit	exposed	6.282	26.667
Little McDonald	exposed	5.896	31.467
Wollaston*	reference	4.884	0.000
Indigo	reference	8.337	93.333
First	reference	7.600	39.467
Fredette	reference	11.805	37.333
Zimmer	reference	8.169	13.333

\* No detectable respiration